THE BRITISH VETERINARY JOURNAL

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EDITORIAL

CAMBRIDGE

THOSE who manned the primitive fort, Caer Grant (the fort by the River Grant) which was sited at the northern end of the modern town of Cambridge could never have visualised that from their small domain a great city, the capital of the fens, would grow and a University develop which would win renown throughout the world. In course of time Caer Grant became Grantanceaster, Grant-bryege and finally Cambridge. Apparently the River Cam did not provide the stimulus for the city's name, but the reverse was the case. Cambridge is old, very old, much older than her ancient University. In spite of their age there are no signs of senility about either town or University. The town is the centre of one of the most progressive and prosperous agricultural areas and the University is a vital seat of learning and nursery of academic youth. Little is known of the origin of the University. Those who breathe the air of another ancient centre of learning, might say, and with some justification, that Cambridge was born of Oxford. We know that in 1209 some scholars from Oxford migrated to Cambridge. We do not know the reason why they deserted their Alma Mater and at this time no useful purpose would be served by delving into the murky past of the "other place". If Cambridge University was born of Oxford her lineage is of the best.

In those days the search for knowledge was pursued with vigour, and it was not long before the number of those who sat at the feet of the scholars at Cambridge was of considerable size. These scholars soon made their presence felt, for all their time was not devoted to academic pursuit. King Henry III found it necessary to bring them under control and at his behest injunctions specifying the penalties and punishments for disorderly conduct were issued. There were

frequent altercations between the townfolk and the students. All work and no play is not good for anyone and the students followed this maxim to the full. Fighting, wounding, breaking and entering and even murder were not uncommon occurrences. The founding of the Colleges, of which Peterhouse (1284) was the first, brought a greater measure of discipline than could be attained by any regal injunctions. As the years passed by more and more Colleges came into being, Cambridge's culture and erudition spread far and wide. Almost every successive generation left its indelible mark in stone and brick, and beautiful buildings register the passage of time and the changes in architectural art. Each College has its own history and the buildings reflect in taste and design the tenor of the requirements throughout the centuries. King's College Chapel, conceived by King Henry VI, sparkles more brilliantly with each succeeding year. It would be interesting to speculate whether the modern boxlike utilitarian University Library will mellow and mature in like manner so as to elicit the adulation of visitors in years to come. Cambridge has so much to show that a visitor could be forgiven if he were to steal away surreptitiously from the more mundane, albeit academic, occasions which called him there.

The organisers of the Congress probably had this matter in mind when they planned the programme. It is an ambitious one and should give profit and interest to workers in every branch of the profession. Whilst in search of beauty and culture let us not forget the exhibition. Congress time is one of the rare occasions when we can make personal contact with our good friends who provide the drugs, the books and the tools with which we work. The exhibitors go to great trouble to show their wares, new productions are put on view and the attendants welcome the opportunity to give first-hand information. In recent years the attendance in the exhibition section has tended to decline. This year let us show the exhibitors that encouragement which will ensure the continuation of a very useful and traditional part of the Congress.

THE ANNUAL B.V.A. CONGRESS, 1957, AT CAMBRIDGE

August 25th to 31st

PROGRAMME OF PAPERS

Monday, Aug. 26th	MORNING		AFTERNOON		
9.30–10.45	11.0	11.30–12.45	2.15–3.25	3.45–4.55	5.0–6.30
Official Opening of Congress Opening of Trade Exhibition	Council Meeting (*Arts School*)	**Presidential Address,** E. R. Callender Annual General Meeting of the B.V.A. (*Lecture Theatre, Department of Zoology*)	Immunological phenomena in parasitic diseases *Author :* Dr. E. J. L. Soulsby *Openers :* Dr. A. M. Dunn K. B. Sinclair *Chairman :* Dr. E. L. Taylor (*Lecture Theatre, School of Veterinary Medicine*)	The general examination of the bull for soundness with special reference to the assessment of fertility *Author :* P. G. Hignett *Opener :* L. E. A. Rowson *Chairman :* C. T. Murphy (*Lecture Theatre, School of Veterinary Medicine*)	**Tour of School of Veterinary Medicine**
			Tour of Animal Health Trust Equine Research Station and Canine Centre		

Tuesday, Aug. 27th	MORNING		AFTERNOON		
9.15–10.30	11.0–11.45	12.0–12.45	2.15–3.25	3.45–4.55	5.30–6.45
Myopathic conditions in animals *Author :* Dr. K. L. Blaxter *Opener :* Dr. J. N. Walton (Myopathy in Man) *Chairman :* Dr. W. R. Wooldridge (*Arts School*)	**Plenary Session** **Collateral hazards from crop spraying** *Author :* Professor Sir Solly Zuckerman, F.R.S. *Chairman :* E. R. Callender (*Lecture Theatre, Department of Zoology*)	**Research problems in animal reproduction** *Author :* Dr. T. R. R. Mann, F.R.S. *Chairman :* W. C. Miller (*Lecture Theatre, Department of Zoology*)	Bacterial endocarditis in cattle *Author :* E. T. Rees Evans *Openers :* P. J. N. Pinsent Dr. S. D. Elliott *Chairman :* Dr. A. W. Stableforth (*Lecture Theatre, School of Veterinary Medicine*)	Mucosal disease *Author :* R. A. Huck *Openers :* A. L. Wilson Dr. A. O. Betts *Chairman :* C. H. Downe (*Lecture Theatre, School of Veterinary Medicine*)	**Films** (*Arts School*)
Oedema disease of swine *Author :* J. F. Timoney *Opener :* M. K. Lloyd *Chairman :* D. F. Oliver (*Arts School*)			**Tour of School of Veterinary Medicine**	**Tour of School of Veterinary Medicine**	

Wednesday, Aug. 28th	MORNING		AFTERNOON		
9.15–10.30	11.0–11.45	12.0–12.45	2.15–3.25	3.45–4.55	5.10–6.15
Physiological foundations of milk production and lactational disorders *Author :* Dr. B. A. Cross *Opener :* Dr. A. Marr *Chairman :* Professor E. C. Amoroso, F.R.S. (*Arts School*)	**Plenary Session** **Veterinary and human medicine** *Author :* Professor A. L. Banks *Chairman:* Professor L. P. Pugh (*Lecture Theatre, Department of Zoology*)	**Studies of the normal animal in relation to disease** *Author :* Sir William Slater *Chairman :* J. N. Ritchie (*Lecture Theatre, Department of Zoology*)	Respiratory diseases of sheep *Author :* A. J. Stevens *Opener :* G. R. Smith *Chairman :* D. Cameron (*Arts School*)	Enterotoxaemia of sheep *Authors :* Dr. J. J. Bullen and Mrs. Irene Batty *Opener :* W. T. Rowlands *Chairman :* Dr. K. C. Sellers (*Arts School*)	**Films** (*Arts School*)
The treatment of fractures in farm animals *Author :* J. Hickman *Opener :* A. C. Shuttleworth *Chairman :* Professor A. Messervy (*Arts School*)			Some problems of reproduction in the mare *Author :* F. T. Day *Opener :* C. S. Haselden *Chairman :* F. G. Waddington (*Arts School*)	Convulsive syndromes in new-born foals *Authors :* L. W. Mahaffey and P. Rossdale *Opener :* Dr. A. C. Fraser *Chairman :* P. J. Mitchell (*Arts School*)	

Thursday, Aug. 29th	MORNING		AFTERNOON		
9.15–10.30	11.0–11.45	12.0–12.45	2.15–3.25	3.45–4.55	5.15–6.45
Some diseases of sucking pigs *Author :* Dr. R. F. W. Goodwin *Opener :* H. I. Field *Chairman :* Professor H. G. Lamont (*Arts School*)	**Plenary Session** **The role of immunology in biology and medicine** *Author :* Dr. R. R. A. Coombs *Chairman :* Sir Alan Drury, F.R.S. (*Lecture Theatre, Department of Zoology*)	**Some factors which may influence the aetiology of diseases of the young** *Author :* Professor R. Lovell *Chairman :* Professor W. I. B. Beveridge (*Lecture Theatre, Department of Zoology*)	Studies in Scrapie *Author :* A. C. Palmer *Opener :* Dr. W. S. Gordon *Chairman :* Dr. R. E. Glover (*Arts School*)	The natural history of parasitic bronchitis *Authors :* Dr. W. F. H. Jarrett Dr. W. I. M. McIntyre F. W. Jennings and Dr. W. Mulligan *Opener :* W. H. Parker *Chairman :* J. J. Low (*Arts School*)	Tour of School of Veterinary Medicine
Present-day problems in veterinary therapeutics *Author :* Professor G. F Boddie *Opener :* A. H. Dawson *Chairman :* J. McConnachie Ingram (*Arts School*)			Films (Small animals) (*Arts School*)	Pathological differentiation of the diseases of the central nervous system of the pig *Author :* J. T. Done *Opener :* J. B. M. Gellatly *Chairman :* E. Cotchin (*Arts School*)	
		Visit to Bayer Biological Institute 1 p.m. (limited to 56)			

Friday, Aug. 30th	MORNING		AFTERNOON		
9.15–10.30	11.0–11.45		2.15–3.25	3.30–4.40	5.40–6.30
Some respiratory diseases of pigs *Author :* P. Whittlestone *Opener :* W. A. Noble *Chairman :* Dr. W. S. Gordon (*Arts School*)	**Plenary Session** **Some clinical observations on the radiotherapy and chemotherapy of malignant tumours in dogs** *Authors :* Professor J. S. Mitchell, F.R.S., and I. A. Silver *Chairman :* Professor T. J. Bosworth (*Lecture Theatre, Department of Zoology*)		The diagnosis and surgical correction of some abnormal stifle conditions in the dog (with film) *Author :* W. B. Singleton *Openers :* M. Young K. M. Dyce *Chairman :* R. N. Phillips (*Lecture Theatre, School of Veterinary Medicine*)	The training, management and employment of various categories of dogs used by the Army *Author :* J. H. Wilkins *Opener :* N. Liakoff *Chairman :* S. F. J. Hodgman (*Lecture Theatre, School of Veterinary Medicine*)	Demonstration of Dog Handling by the R.A.V.C. (*School of Veterinary Medicine*)
The relationship of soundness and conformation in the horse *Author :* H. W. Dawes *Opener :* E. Brayley Reynolds *Chairman :* E. Wilkinson (*Arts School*)				**Tour of Houghton Poultry Research Station**	
Saturday, Aug. 31st	Closing meeting of Congress.		First meeting of the Council, 1957–8.		

Congress Coach and Bus Transport Arrangements

BUSES have been booked to take visitors to the School of Veterinary Medicine for sectional meetings and tours. A weekly ticket inclusive of all these journeys can be obtained from the B.V.A. office. Separate tickets will be issued for other tours.

In general, transport leaves and returns to Tennis Court Road and Downing Street, which roads adjoin each other, with Bridge Street as an intermediary stopping place. Tennis Court Road and Downing Street are convenient for those attending meetings at the Arts School and Department of Zoology and for those staying at the University Arms Hotel, Downing College, Royal Hotel, Garden House Hotel, New Hall, and the Lion Hotel. Northampton Street and Bridge Street stopping points are next to Magdalene College and close to the Blue Boar Hotel.

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GENERAL ARTICLES

LABORATORY TRIALS WITH AN AVIANISED RINDERPEST VACCINE

By S. E. PIERCY and M. A. WITCOMB

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THE control of rinderpest in East Africa is achieved mainly by the use of two live vaccines containing virus attenuated in the goat and rabbit respectively. The first, usually referred to as K.A.G. (Kabete Attenuated Goat Virus), is designed for the immunisation of indigenous Zebu cattle, although in recent years a number of European farmers have found it suitable for low-grade European/Zebu type crosses. The second, lapinised vaccine, has with few exceptions proved a safe and effective agent for the protection of pure-bred and high-grade exotic breeds of cattle and for high yielding dairy cows.

Lapinised vaccine was developed by Nakamura (1938, 1940-51) and his associates in 1935 with the object of elaborating an immunising agent which would be safe to use in the highly susceptible breed of Japanese Black cattle. Even this product, mild though it is, causes unacceptably severe reactions in certain hypersusceptible breeds, as for example in Korea, with the result that further attenuation became necessary and was achieved by adapting lapinised virus to embryonated eggs (Nakamura and Miyamoto, 1953, Reisinger *et al.*, 1954, Nakamura *et al.*, 1955).

In East Africa, lapinised vaccine, when used in European and Zebu breeds of cattle, causes little or no clinical reaction or thermal response. Severe reactions may occur occasionally in certain types of exotic breeds (Brown, Scott and Brotherston, 1955). The immunity conferred in fully susceptible animals appears to be of two years' duration or more (Piercy, 1953). This vaccine has a number of distinct disadvantages. It is costly to produce, the virus is considered to be very fragile (Scott and Brotherston, 1952), and the lack of thermal response in inoculated cattle precludes a ready assessment of its potency and viability in the field. Furthermore, in spite of good management, a colony of rabbits can sometimes be decimated or severely reduced in numbers by outbreaks of coccidiosis or Pasteurellosis, events which may seriously jeopardise routine vaccine production. It seemed wise, therefore, to reconsider the merits of avianised rinderpest vaccine for the immunisation of cattle in East Africa. Firstly, it was hoped that avianised vaccine would prove to be a valuable and readily available substitute should the production of lapinised or caprinised vaccines ever fail. Secondly, it was anticipated that it might lack some of the defects referred to in the production of lapinised vaccine. Thirdly, it seemed possible that avianised vaccine might prove useful in circumstances when neither K.A.G. nor lapinised vaccines are entirely appropriate.

The earliest record of attempts to adapt the rinderpest virus to embryonated eggs is that of Kunert (1938) who claimed to have recovered virus after four passages through eggs. The first serious attempt to elaborate an avianised

vaccine was made by the Grosse Isle workers (Shope *et al.* 1946). They produced convincing evidence to show that the immunisation of highly susceptible cattle against rinderpest by means of a dried egg vaccine is well within the bounds of practical possibility. This work was pursued by workers at Kabete (Hudson, 1947; Danks, 1950) with rather disappointing results. Meanwhile the use of a mild attenuated strain of virus had obvious application amongst the highly susceptible cattle of the Far Eastern region, and Cheng, Chow and Fischman (1948) reported the successful use in China of avianised vaccine prepared by the Grosse Isle group, and prepared by themselves from an egg-adapted strain of bovine virus obtained from Kabete. Finally, Ishii and his associates (1953) in Japan reported the successful adaptation of their own laboratory strain of bovine virus to embryonated eggs. In some cases, however, this strain proved to be still too virulent for certain types of hyper-susceptible cattle, necessitating the use of the even milder strain of avianised-lapinised virus (Nakamura, 1954).

In East Africa, certain field trials with the Grosse Isle strain of avianised virus were made in 1945 (Hudson, 1947), with moderately good results. So far as can be ascertained, records have never been published in full. Since then interest in this product has waned, due undoubtedly to the successful results obtained with caprinised and lapinised vaccines. Nevertheless, the conclusions of the F.A.O., Nairobi Rinderpest Meeting (1949) make interesting reading. Thus "the present knowledge indicates that the avianised virus vaccine is the most suitable and safe immunizing agent for the protection of hyper-susceptible animals" and "there is no doubt that the living attenuated virus vaccines are the cheapest form of prophylactic. The cost of each of these will vary according to local conditions, but it is considered that, when fully developed, the bulk production of avianised vaccine would be the cheapest, but further investigation is required to determine the place of lapinized virus vaccine." There are, then, several good reasons for a thorough re-examination of the use of avianised vaccine amongst cattle in East Africa.

Materials and Methods

(i) *Virus strain.*

This was obtained as dried egg-embryo spleen, labelled "Strain BA, passage number A-Y 227," through the courtesy of Dr. J. Nakamura, Director of the Nippon Institute of Biological Science, Tokyo. Sent by air, packed in ice, the virus arrived 12 days after despatch and was then stored at −25° C. Two weeks later the contents of one ampoule were reconstituted in nutrient broth, passed through eggs by the yolk sac route and the presence of virus in embryonic spleen, embryo and yolk sac demonstrated by cattle inoculations.

The strain of virulent bovine virus had been adapted by Ishii and his associates (1953) to the chorio-allantoic membrane and yolk sac of embryonated eggs. This was the strain used to begin the historic series of rabbit passages in 1935 (Nakamura, 1954) (Nakamura *et al.* 1938). According to Cheng and Fischman (1948) it had been carried in the laboratory in its virulent state for 10 years, before that. Its history (Nakamura, 1955) is as follows:—

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Bovine virus—M30-B1-R2-M6-B1-M12-B1-M9-B1-M26-
B1-M5-B1-M1-Y44-B1-M1-Y193.

M = Passage on the chorio-allantoic membrane.
B = Passage through cattle.
R = Passage through rabbits.
Y = Passage through the yolk sac.
Numeral = Number of serial passages.

When received by us, therefore, strain BA was in its 237th yolk-sac passage.

(ii) *Technique.*

Standard procedures for the inoculation, harvesting and emulsification of eggs were used throughout (Piercy, 1952). Unless otherwise stated in the text, 5-day old embryonated eggs were inoculated into the yolk sac with 0.5 ml. of a 10 per cent suspension of infective material. The eggs were incubated at 95° F. and usually harvesting took place on the 8th day after inoculation.

(iii) *Cattle.*

The animals used for the demonstration and titration of avianised virus were, for the most part, medium grade European/Zebu type crossbred steers although from time to time animals with considerably more native or European blood, as the case may be, were encountered. They all came from areas in which rinderpest has been absent for many years and from farms where rinderpest vaccines are deliberately withheld so that animals are available for sale as "rinderpest susceptibles." Cattle used for tests with avianised vaccine were housed in isolation, in sheds where no virulent virus has ever been used, and attended to by a cattleman who has no other responsibility. The possibility of such cattle coming into contact with virulent virus can be excluded.

(iv) *Drying and storage of seed material.*

Infected egg material, after disintegration in mincer tubes or in an Atomix* blender, was dried in ampoules on an Edwards† L.T.5 freeze-drying machine, sealed *in vacuo* and stored at −25° C.

(v) *Cattle challenges.*

Cattle were challenged either with a standard dose of virulent Kabete "O" virus, or with a field dose of K.A.G. virus. Kabete "O" virus is a virulent laboratory strain which has been in use at the Veterinary Research Laboratory, Kabete, and at this Institute for the past 40 years. The standard challenge dose contains not less than 1,000 M.I.D. K.A.G. virus was commonly used when estimating, for example, the titre in cattle of avianised virus maintained under different conditions of storage. Immunity or susceptibility can be assessed readily by the presence or absence of thermal reactions. By adopting this method where appropriate, it was possible to avoid much of the heavy expense inseparable from the use of virulent challenge virus.

(vi) *Assessment of Temperature Reactions.*

In this particular climate, diurnal fluctuations of body temperature are very marked. A rise of two degrees during the day is by no means uncommon, depending upon weather conditions. For this reason it is the usual practice at this institute

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to record the temperatures of experimental animals on mornings only. For purposes of assessing the clinical reaction of cattle receiving avianised virus, a two degree rise above the animal's mean normal morning temperature was considered a positive thermal response.

Results

(i) *Estimations for the presence of virus in eggs.*

The contents of one ampoule of dried embryo spleen (as received from Japan), were reconstituted in 10.0 ml. nutrient broth and inoculated into a number of eggs. Eight days later embryo spleens, whole embryos and yolk sacs were harvested separately and dried. The materials in a number of ampoules were then reconstituted and tested in cattle, by subcutaneous inoculation, for the presence of virus (table 1).

TABLE 1
Tests in cattle for the presence of virus in a number of egg constituents

Animal	Material	Temperature Reaction	Challenge with K.A.G. virus
846	2.0 ml. dried embryo spleen, ...	Yes	Immune
844	diluted 1/100	No	,,
843	2.0 ml. dried yolk-sac, ...	Yes	,,
847	diluted 1/10	No	,,
842	2.0 ml. dried whole embryo, ...	Yes	,,
845	diluted 1/10	Yes	,,

A titration of freshly harvested wet embryo from eggs inoculated and incubated as before was now made (table 2).

TABLE 2
Titration in cattle of wet embryo

Animal	Material	Dilution	Temperature reaction	Challenge
1003	1.0 ml. embryo	Undiluted	Yes	Immune*
1100	,, ,,	,,	Yes	,, *
1104	,, ,,	10^{-2} ,,	Yes	,, *
1078	,, ,,	,,	No	,, *
1077	,, ,,	10^{-3} ,,	Yes	,, *
1103	,, ,,	,,	Yes	,, *
1102	,, ,,	10^{-4} ,,	Yes	,, †
1101	,, ,,	,,	Yes	,, †
1149	,, ,,	10^{-4} ,,	No	,, *
1148	,, ,,	,,	Yes	,, *
1145	,, ,,	10^{-5} ,,	Yes	,, *
1142	,, ,,	,,	Yes	,, *

* Challenged with K.A.G. virus.
† Challenged with Kabete "O" virulent virus.

These results show that virus sufficient to immunise cattle was present in dried embryo spleen, yolk sac and embryo; and in wet embryo to a titre of at least 10^{-5}.

To obtain an immediate indication whether there was good distribution of virus throughout the egg, freshly harvested whole egg was emulsified and titrated with the following results (table 3).

TABLE 3
Titration in cattle of wet, whole egg

Animal	Material	Dilution	Temperature reaction	Challenge with Kabete O virulent virus
1151	1.0 ml. whole egg	10^{-3}	Yes	Immune
1147	,, ,, ,,	,,	Yes	,,
1144	,, ,, ,,	10^{-4}	No	,,
1150	,, ,, ,,	,,	No	,,
1143	,, ,, ,,	10^{-5}	Yes	,,
1146	,, ,, ,,	,,	No	,,

The above results suggest that there was good distribution of virus throughout the egg since whole egg immunised cattle at a dilution of 10^{-5}, the same figure as was obtained for wet embryo. It is of interest to note that one good temperature reaction was obtained at the highest dilution.

(ii) *Experimental vaccine production.*

Batch I. There was now good evidence that egg material contained virus to a high titre and since a prime aim of this work was to investigate the production of a dried vaccine on a large scale, the mechanics of bulk drying were explored.

Using 238th passage dried yolk-sac as seed, 10 eggs were inoculated in the usual manner with the object of invigorating the organism by the interpolation of one wet passage between seed and vaccine inoculum. Eight days later a number of live embryos were harvested, emulsified in an equal quantity of normal saline and inoculated to a further group of 60 eggs. On the 8th day post-inoculation, 20 live eggs were harvested and the whole contents emulsified in a Waring blender. The material was then dispensed into ampoules in 2.0 ml. amounts. After shell-freezing, the ampoules were dried on a Hale-Walker type dry-ice vacuum machine (Hale and Walker, 1946). Drying continued overnight at a vacuum of 0.02 mm. Hg. The next morning the ampoules were removed, the necks constricted so as to allow final sealing *in vacuo*, and replaced on the machine for a period of 2 hours secondary drying. The ampoules were sealed with an oxy-acetylene flame and stored at −25° C. The moisture content of the dried vaccine was just below one per cent.

One gramme of dried vaccine was diluted in tenfold series in normal saline. 1.0 ml. amounts of these dilutions were given subcutaneously to cattle within an hour or so of reconstitution, using 4 animals per dilution. Three weeks later the animals were challenged with Kabete " O " virulent virus. The results are recorded in table 4.

These results show that two of four animals given vaccine at a dilution of 10^{-5} resisted infection.

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TABLE 4

The immunisation of cattle with dried Avianised Rinderpest Vaccine. Experimental Batch I

Animal	Material	Dilution	Temperature reaction	Challenge
1281	Dried whole egg	10^{-3}	Yes	Immune
1399	,, ,, ,,	,,	No	,,
1385	,, ,, ,,	,,	Yes	,,
1396	,, ,, ,,	,,	Yes	,,
1295	,, ,, ,,	10^{-4}	Yes	,,
1384	,, ,, ,,	,,	Yes	,,
1317	,, ,, ,,	,,	No	,,
1304	,, ,, ,,	,,	Yes	,,
1377	,, ,, ,,	10^{-5}	No	,,
1405	,, ,, ,,	,,	No	,,
1402	,, ,, ,,	,,	No	Susceptible
1407	,, ,, ,,	,,	No	,,

Batch II. The seed material used for batch II was infected embryo spleen stored wet for 7 days at −25° C. A 1/250 suspension was inoculated into 34 five-day old eggs. On the 8th day all the surviving eggs, together with 4 eggs that had died that day, were harvested, emulsified and dispensed into ampoules in 2.0 ml. amounts. Drying was effected on an Edwards 3 P.S. machine.

Batch II vaccine was titrated by the same technique as before with the case of two animals per dilution and challenging with K.A.G. virus. One of the two animals given vaccine diluted to 10^{-4} resisted infection, one log lower than with batch 1.

Batch III. This was made in the same way as for batch I except that the seed used was dried whole egg. Drying was again carried out on an Edward 3 P.S. machine. The titration, using 4 animals per dilution, revealed that half were immunised at $10^{-3.5}$.

Since the results with batches II and III were inferior to batch I, an examination of techniques was made. The only point that appeared to merit further investigation was that whereas batch I was dried immediately after harvesting on a Hale-Walker type drying machine, batches II and III were dried on an Edwards 3 P.S. machine which, for domestic reasons, necessitated an interval of storage in the wet state at −25° C. between harvesting and drying. Bearing this in mind a 4th batch was made.

Batch IV. Particular care was taken to begin drying (this time on an Edwards LT.5 machine) immediately after harvesting. When titrated in cattle using dilutions of 10^{-3} to 10^{-5}, all animals were immune to challenge and no end-point was reached. This result seemed to suggest that rapid drying after harvesting might be a factor of importance and the next batch was prepared accordingly.

Batch V. Using dried whole egg as seed, and interpolating one wet passage between seed and vaccine inoculum, 60 eggs were inoculated. Twenty-two eggs dying on the 6th, 7th and 8th days after inoculation were collected and these, together with eggs still alive on the 8th day, were emulsified, pooled and dried on

the Hale-Walker apparatus. After 24 hours primary drying the material was removed from the bottles, bulked and ground up in a large mortar, and then re-dispensed in 1 g. amounts into ampoules. Secondary drying and sealing were carried out on the secondary system of an Edwards LT.5 machine. Titrations in cattle were carried out as before using 1.0 ml. of dilutions made from 1 g. of dried vaccine. Three weeks after vaccination the animals were challenged with Kabete "O" virulent virus (table 5).

TABLE 5
The immunisation of cattle with dried avianised rinderpest vaccine, experimental batch V

Animal	Dilution	Temperature reaction	Challenge (Kabete "O" virulent virus)
2644	10^{-2}	Yes	Immune
2652	,,	Yes	Immune
2661	10^{-3}	Yes	Immune
2655	,,	Yes	Immune
2651	10^{-4}	No	Immune
2656	,,	No	Susceptible
2653	10^{-5}	Yes	Immune
2660	,,	No	Susceptible
2663	10^{-6}	Yes	Immune
2641	,,	Yes	Immune
2667	10^{-7}	No	Susceptible
2659	,,	No	Susceptible
2647	10^{-8}	No	Susceptible
2645	,,	No	Susceptible

TABLE 6
Dried avianised rinderpest experimental vaccines, batches I-V. Summary of titrations

Batch	Seed	Harvest	Method of Drying	Thermal response to vaccination*	Challenge material	Immunising titre.
I	Yolk-sac	8th day live eggs	Hale-Walker	60 per cent	Kabete O virulent virus	10^{-5}
II	Embryo spleen	8th day live & dead eggs	Edwards 3PS	66 per cent	K.A.G. virus	10^{-4}
III	Whole egg	8th day live eggs	Edwards 3PS	25 per cent†	K.A.G. virus	$10^{-3.5}$
IV	Whole egg	8th day live eggs	Edwards LT5	75 per cent	K.A.G. virus	10^{-5} (No end point)
V	Whole egg	6-8th day dead eggs & 8th day live eggs	Hale-Walker & Edwards LT5	87.5 per cent	Kabete O virulent virus	10^{-6}

* That is in cattle successfully immunised.
† Only 4 animals involved.

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(iii) *Keeping properties of reconstituted vaccine.*

Since the immunising ability of the dried vaccine appeared satisfactory, a number of experiments was now planned to estimate the life of virus reconstituted from the dried state and maintained for varying periods at different temperatures. The temperatures chosen were 20° C., 30° C. and 40° C., representing ideal, common and very adverse field conditions respectively. The method used was to select one or two ampoules of vaccine at random, weigh out one gramme from the pooled contents, and make tenfold dilutions in normal saline, ranging from 10^{-2} to 10^{-5}. The tubes of reconstituted vaccine were then maintained at the chosen temperatures for varying periods of time, after which two animals per dilution were inoculated with 1.0 ml. of material. After periods of two to three weeks all animals were challenged with field doses of K.A.G. virus. The results are recorded in table 7.

TABLE 7
The effect of time and temperature on reconstituted avianised rinderpest vaccine

Material	Maintained at		Thermal response to vaccination	Immunising titre	Fall in titre from original
	Temp.	Time			
Batch I	20°C	6 hours	50 per cent	10^{-5}	0 log
Original	,,	24 ,,	33 ,, ,,	10^{-3}	2 ,,
50 per cent	30°C	2 ,,	83 ,, ,,	$10^{-4.5}$	½ ,,
immunising	,,	6 ,,	80 ,, ,,	10^{-4}	1 ,,
titre	40°C	1 hour	80 ,, ,,	10^{-4}	1 ,,
10^{-5}	,,	3 hours	66 ,, ,,	10^{-3}	2 ,,

Summary of Results.

(*a*) 6 *hours at* 20° *C.* (68° *F.*). No appreciable loss of titre occurred in reconstituted vaccine maintained for 6 hours at 20° C.

(*b*) 24 *hours at* 20° *C.* (68° *F.*). The end point of protection was at 10^{-3}, all animals at higher dilutions proving susceptible to challenge. Thus, maintaining reconstituted vacine at 20° C. for 24 hours resulted in a two log drop in titre.

(*c*) 2 *hours at* 30° *C.* (86° *F.*). This temperature represents a common shade heat encountered in the field during cattle immunisation programmes and the results of this particular experiment, therefore, are particularly important. After 2 hours an apparent slight drop in titre of the reconstituted vaccine had occurred. But although neither of the animals receiving the 10^{-5} dilution were immunised, it must be remembered that the vaccine when originally tested only protected two out of four animals at this dilution. Had more animals been used, therefore, it is possible that a proportion of them would have been protected by the 10^{-5} inoculation.

(*d*) 6 *hours at* 30° *C.* (86° *F.*). The above observation is supported by the fact that after 6 hours at 30° C. a fall in titre of only 1 log had occurred.

(*e*) 1 *hour at* 40° *C.* (105° *F.*). This temperature was chosen as representing the hottest shade conditions likely to be encountered during the immunisation of cattle in East Africa. A period of one hour was chosen since it was considered unlikely that reconstituted vaccine would maintain its titre for much longer at this high temperature. The results show that a loss of titre had, in fact, begun to occur within this time. Thus the end point was at a dilution of 10^{-4}, a fall in titre of

1 log. After three hours, a further log drop occurred.

(iv) *Keeping properties of dried vaccine.*

When it became known that the keeping properties of reconstituted vaccine were good, a similar examination of dried vaccine was planned. At this time, unfortunately, the supply of experimental amimals became restricted and only a limited investigation was possible. It was thought that a start should be made by examining the viability of dried vaccine when maintained at a comparatively unfavourable temperature such as 30° C. (86° F.), the kind of thermal condition that might be encountered should a consignment of vaccine go astray in a hot climate, or be accidentally left in the back of a motor vehicle. Accordingly, a number of ampoules of dried vaccine were placed in an incubator set at 30° C. for a week. After this time, one gramme of material was weighed out, reconstituted and tenfold dilutions made as before. Two cattle per dilution were inoculated with the reconstituted vaccine without delay. After a three weeks interval they were challenged with a field dose of K.A.G. virus (table 8).

TABLE 8
The effect of time and temperature on dried avianvised rinderpest vaccine.

Material	Maintained at		Thermal response to vaccination	Immunising titre	Fall in titre from original
	Temp.	Time			
Batch I	30°C	7 days	66 per cent	10^{-4}	1 log
Original immunising titre 10^{-5}	20°C	10 ,,	50 ,, ,,	$10^{-3.5}$	1.5 ,,
	20°C	44 ,,	—	$<10^{10-3}$	>2 ,,
	—25°C	9 months	50 per cent	$10^{-4.5}$	0.5 ,,

Comparing these results with those obtained when dried vaccine was reconstituted and given at once to cattle, it is seen that a one log drop in titre occurred after 7 days storage at 30° C. (86° F.), a protection end point being obtained at the 10^{-4} dilution.

Meanwhile, a number of ampoules of dried vaccine had been placed on the bench at room temperature (20° C.). After 10 and 44 days respectively this material was tested in the same way with the results as shown in table 8.

A 1.5 log drop in titre occurred after 10 days storage at room temperature and a greater loss than this after 44 days. Loss of virus of this magnitude at room temperature had not been expected and this experiment must obviously be repeated. In view of the good result obtained at 30° C. for 7 days, it is hoped that the comparatively poor result at room temperature can be related to some technical shortcoming such as loss of vacuum from the ampoules tested.

Finally, an ampoule of batch I vaccine was reconstituted after 9 months' storage at −25° C. It was tested in cattle as before, using 2 animals per dilution (table 8). A drop in titre of half a log was revealed.

Discussion

As Hudson and Wongsongsarn (1950) remark, "The ideal attenuated virus for immunisation is probably one that will cause a mild temperature reaction without

other clinical symptoms. Future work on virus attenuated in the chick-embryo or by other means may lead to the development of such a strain." The results of the work reported in this article encourage the belief that avianised vaccine could play an important part in the immunisation of cattle in East Africa against rinderpest. There are several distinct advantages inherent in its use.

In the first place, avianised vaccine is simple and economical to produce and avoids the administrative and technical difficulties inseparable from the use of live animals such as the goat and rabbit. Fertile eggs constitute a readily available, sterile medium of standard composition for the growth of virus. In our hands the egg vaccine had an immunising titre at least as good as caprinised vaccine and much superior to lapinised vaccine. With dried whole egg the immunising titre of batch I was 10^{-5} and of batch V 10^{-6}. Allowing a margin for field use of 100 ID_{50}, 1 gramme of these vaccines would provide 1,000-10,000 field doses. Adopting a more severe calculation by regarding 10^{-4} as the complete immunising end-point, and allowing a margin of 100 M.I.D., one gramme provides at least 100 field doses. Since one whole egg yields about 10 grammes of dried material, 1,000 field doses are obtained per harvested egg and 10,000 doses from a dozen eggs (allowing for two infertiles). The cost of one dozen fertile eggs is rather less than the cost of one 4-month old rabbit from which 500-1,000 doses of lapinised vaccine are usually obtained. The cost of producing one cattle dose of avianised vaccine, therefore, may prove to be less than one tenth that of lapinised vaccine.

Secondly, the fact that temperature reactions were recorded in a high percentage of animals receiving avianised virus is most valuable as an aid to the ready assessment of the viability and potency of vaccine used in the field. Over 70 per cent. of immunised animals showed a significant thermal response. This figure may be compared with the results obtained by Hale and his colleagues (1946) who state "approximately 76.27 per cent. of the calves will demonstrate a definite temperature rise to 104° F. or higher, following administration of the avianised vaccine." It has always been a major disadvantage of lapinised rinderpest vaccine that there are no ready means of assessing a clinical response. In this connection two quotations from an article by Scott and Brotherston (1952) are apposite. "In Kenya lapinised rinderpest vaccine, when inoculated into European and grade cattle, causes no reaction in the majority of cases; thus one cannot easily detect whether the lapinised vaccine is viable or not, as one can when using goat-adapted rinderpest virus which produces a definite clinical reaction. It is, therefore, imperative for field officers to follow strictly the instructions regarding the handling of lapinised vaccine and, similarly, it is important that a large and practical safety margin be allowed," and later "It is emphasized that because of the absence of a marked thermal reaction in the majority of cattle inoculated with lapinised vaccine, great care is required to ensure that potent material is inoculated." To the officer responsible for immunising a farmer's cattle, and to the owner himself, it is always a distinct satisfaction to know that the animals concerned are reacting to vaccination, thus demonstrating that the inoculations were properly carried out with potent material. Using avianised vaccine, and providing that more than one or two animals are inoculated at any one time, a record of temperature reactions of a group of 10 animals will provide this assurance.

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The third important point for discussion is the keeping properties of avianised vaccine. As regards dried material, there is already ample evidence provided by the Grosse Isle, Chinese and Japanese workers that properly dried virus stored at temperatures below freezing point will remain potent for more than one year. In our own experiments, dried vaccine dropped only one log in titre after 7 days at 30° C. (86° F.), and half a log after a period of 9 months at −25°C.

The most important point, however, is the effect of time and temperature on reconstituted vaccine for on this depends the precautionary measures that must be observed in the field. After 6 hours at room temperature, no appreciable loss in titre occurred. After 2 and 6 hours respectively at 30° C. (86° F.), not more than one log loss was recorded in either case. And at 40° C. (105° F.), a one log drop in titre occurred after 1 hour and two logs after 3 hours.

These results are rather better than those obtained by the Grosse Isle workers, possibly because the techniques of production used may represent an advance since the war years, and perhaps because the experiments were conducted on a more comprehensive and rational basis of titrations. There are no detailed protocols on this particular subject in the Grosse Isle papers but Hale and his colleagues (1946) state that " Exposure of the fluid vaccine to room temperature (22° C.) for twenty-four hours or more reduces the potency of the vaccine, and after seventy-two hours it has been shown to be completely inactivated " and " it is recommended that no reconstituted vaccine be used after a twelve-hour period." They also remark that " the exact length of time the attenuated virus may be maintained at this temperature (22° C.) has not been determined, but there is evidence that exposure to room temperature for a period of twenty-four hours will result in complete loss of virus." The latter statement refers to virus contained in mixed allantoic and amniotic egg fluids and one reason why our results at room temperature were so much better (a two log drop in 24 hours) may be because the virus was contained in whole egg and thereby protected by certain colloidal and buffering egg constituents. In this connection the statement of Hale and his colleagues (1946) that " vaccines composed of fluids and embryos apparently withstand exposure to adverse temperatures better than those containing the entire egg contents " cannot be accepted without reservation in view of certain incongruities and the small number of animals used in the experiment to which they refer.

It is of some interest to compare the present results with those obtained for lapinised vaccine by Scott & Brotherston (1952). They found that 0.002 gramme (half a field dose) of reconstituted vaccine was still capable of immunising cattle after 6 hours, but not after 12 hours, when maintained at 20-22° C., and after 1 hour but not after 2 hours when maintained at 37.0-37.8° C. A direct comparison of their results with ours is not easy to make since titrations were not made. For example, the vaccine they used (K.9 batch 60) was originally capable of immunising cattle at a dosage of 0.0002 grammes. A fall of one log in titre would not, therefore, be revealed by their method of examination. Nevertheless, their results, where applicable, bear close resemblance to ours. It is quite evident that when using either lapinised or avianised vaccine in cool conditions, there is an ample margin of time after reconstitution to inoculate, for example, one hundred cattle from one ampoule. In hot conditions, however, for example 90-100° F. in the shade, recon-

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stituted vaccine must be put into cattle within an hour to avoid dangerous deterioration. Alternatively, and this is obviously the correct approach, reconstituted vaccine must be kept reasonably cool, for example by immersing the container in water. Provided this be done when necessary it would seem that Scott & Brotherston's (1952) suggestion that " thirty minutes after reconstitution is the upper safety limit for the vaccine " is unnecessarily generalised and severe if applied under all conditions and may result in the unnecessary discard of perfectly good vaccine or alternatively an undesirable scramble to inoculate a hundred head of cattle in half an hour. Our results show that with avianised vaccine, at all events, when used within the range of shade temperatures most commonly encountered in the field in East Africa (70-90° F.), only a small loss of titre occurs in 2-6 hours which can be offset by selecting a field dilution factor that provides a margin of 100 M.I.D. Since this vaccine is so economical to produce, an ample excess of immunising doses is feasible.

The work described in this article raises another and most interesting point. It is well known that if serial passage with rinderpest virus in eggs is confined to the chorio-allantoic membrane, attenuation is extremely slow. For this reason adaptation to the yolk sac as soon as possible is desirable. When this is achieved nearly all workers emphaise that attenuation proceeds so rapidly that there is a real danger of losing the virus or that it will become too weak for immunising purposes. For example, Hale & Walker (1946) state " The failue of the 58th yolk-sac passage virus to produce immunity is probably due to excessive attenuation. The only alternative is that the virus was lost during transfer between the 53rd and 58th passage level." Similarly, the Kenya Veterinary Department's Annual Report (1947) states that after 25 yolk sac passages " the virus gave little or no protection and passage was discontinued," and later " From the thirty-first passage onwards the yolk sac material gave no protection and the experiment was discontinued." The 1948 report states that " Attenuation was very rapid in the yolk sac and after about 30 yolk sac passages there were no reactions in inoculated animals and no immunity conferred." Daubney (1950) working in Cairo, made a similar observation when he stated " It seems probable that these yolk-sac lines once they are established, pass rapidly from the stage at which they are too virulent to use on any cattle to the stage when they are too attenuated for cattle that possess any degree of natural resistance."

In remarkable contrast to these observations is the fact that the virus used in the present experiments was in its 238th yolk sac passage. Admittedly there was a break at the 44th passage when the virus was passed through one ox but since then there has been an uninterrupted series of 194 yolk-sac passages. Only three possible explanations come to mind. The first is that the Nakamura strain may be peculiarly different from those used by other workers. Or possibly this strain has become " fixed " in its characteristics. Or thirdly it may be that the several instances of extreme attenuation referred to in the above quotations were caused by actual loss of virus due, for example, to inadequate storage facilities. In this connection a remark by Hudson (1947) is not without significance; " One of our main difficulties was the provision of storage facilities for material of each passage so that, should anything go wrong with the direct line, we could return to stored

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material. On Grosse Island material was stored at −60 to −70° C., whereas we can only store at −5° C."

That avianised rinderpest virus, when attenuated to the proper level and correctly prepared as a vaccine, will provide cattle with a serviceable immunity is beyond doubt. So far as our work has gone we appear to have produced a cheap and effective vaccine, possessing good keeping properties and easily capable of mass production. There seems good reason to suppose that it could be used as a very useful alternative to caprinised or lapinised vaccines or even be preferable to either in certain circumstances, for example when caprinised virus is considered to be too virulent and lapinised virus too attenuated for the class of cattle to be immunised. Much further work remains to be done, however. In the first place field trials do not always support laboratory findings. Secondly, whilst the only sign of a clinical reaction in the medium-grade cattle used by us was a thermal response, the effect of this vaccine in high-grade and pure-bred European types of cattle has not yet been estimated. And thirdly, little is known of the duration of immunity although Danks (1950) reported solid immunity in cattle challenged 17 months after inoculation. There seems no reason to doubt, however, that the duration of immunity will be at least as long as that following the use of lapinised vaccine (two years or more) and possibly longer in view of the fact that avianised vaccine is sufficiently strong to cause temperature reactions in a high percentage of animals.

Summary and Conclusions

1. A number of experiments with a Japanese strain of avianised rinderpest virus are described.

2. One gramme of dried whole egg vaccine contained sufficient virus to immunise half the cattle challenged at a dilution of 10^{-6}.

3. Reconstituted whole egg vaccine suffered no appreciable loss of titre when maintained for 6 hours at room temperature (20° C.). After 24 hours at this temperature a 2 log drop occurred.

When maintained at 30° C. (86° F.) for 2 and 6 hours respectively, a drop in titre of not more than 1 log resulted.

At 40° C. (105° F.), the titre fell by 1 log after 1 hour and by 2 logs after 3 hours.

4. Dried whole egg vaccine lost 1 log in titre after maintenance for 7 days at 30° C. (86° F.), one and a half logs after 10 days at room temperature and half a log after 9 months at −25° C.

5. Of all animals immunised with dried vaccine at a dilution of 10^{-3}, which is ten times more dilute than the suggested field dose, 80 per cent. showed a significant thermal response.

6. A method for the production of large quantities of cheap dried avianised rinderpest vaccine is described. Ten fertile eggs infected with virus can provide 10,000 cattle doses each containing 100 M.I.D.

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† W. Edwards & Co. (London) Ltd.

REFERENCES

Brown, C. W., Scott, G. R. and Brotherston, J. G. (1955): *Vet. Rec.*, *67*, 467.
Cheng, S. C., Chow, T. C. and Fischman, H. R. (1948): F. A. O. Agricultural Studies No. 8, 1949. "Rinderpest Vaccines," Washington, U.S.A.
Cheng, S. C. and Fischman, H. R. (1948): *Ibid.*
Danks, W. B. C. (1950): *Ann. Rep. Dept. Vet. Services*, Kenya, 1948. Government Printer, Nairobi.
Daubney, R. (1950): Kabete Veterinary Research Laboratory File.
F.A.O. Agricultural Studies No. 8 (1949): "Rinderpest Vaccines," Washington, U.S.A.
Hale, M. W. and Walker, R. V. L. (1946): *Amer. J. vet. Res.*, *7*, 199.
Hale, M. W., Walker, R. V. L., Maurer, F. D., Baker, J. A. and Jenkins, D. L. (1946): *Ibid.*, 212.
Hudson, J. R. (1949): *Ann. Rep. Dept. Vet. Services*, Kenya, 1947. Government Printer, Nairobi.
Hudson, J. R. (1947): Kabete Veterinary Research Laboratory File.
Hudson, J. R. and Wongsongsarn, C. (1950): *Brit. vet. J.*, *106*, 453.
Ishii, S. and Tsukuda, K. (1953): *Rep. Gov. Exp. Stat. Anim. Hyg.*, *26*, 29.
Kunert, H. (1938): *Dtsch. tierärztl. Wschr.*, *46*, 487. (Abstract in *Vet. Bull.*, *9*, 536, 1939).
Nakamura, J., Wagatuma, S. and Fukusho, K. (1938): *J. Jap. Soc.Vet. Sci.*, *17*, 185 (Japanese original); 25, English Abstract.
Nakamura, J. *et al.* (1940–51): *Jap. J. Vet. Sci.*, *2–12*.
Nakamura, J. and Miyamoto, T. (1953): *Amer. J. Vet. Res.*, *14*, 307.
Nakamura, J. (1954): O.I.E. Conference, Bangkok, February, 1954.
Nakamura, J., Kishi, S., Kiuchi, J. and Reisinger, C. R. (1955): *Amer. J. Vet. Res.*, *16*, 71.
Nakamura, J. (1955): Personal communication.
Piercy, S. E. (1952): *J. comp. Path.*, *62*, 152.
Piercy, S. E. (1953): *Proc. XV Inter. Vet. Cong.*, Stockholm, 1953.
Reisinger, C. R., Mure, C. P. and Lee, N. S. (1954): *Amer. J. Vet. Res.*, *15*, 554.
Scott, G. R. and Brotherston, J. G. (1952): *J. comp. Path.*, *62*, 108.
Shope, R. E. *et al.* (1946): *Amer. J. Vet. Res.*, *7*, Part 2. I–XVI.

A CASE OF BI-LOBED LEFT ADRENAL WITH ABSENCE OF MEDULLARY TISSUE IN A SHEEP

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DURING the routine collection of sheep adrenal glands at the City Abattoir, Edinburgh, for general research purposes, attention was drawn to considerable deviation from the normal in the appearance and measurement of the left gland in one of the carcases.

Bearing in mind the susceptability of adrenals in general to changes brought about by sexual activity, disease processes and nutritional factors, a peculiar bi-lobed appearance of this gland was interesting, and it was decided to make further investigation in the hope that such might be complementary to the work already on hand.

Description

A. General Remarks.

From a superficial examination of the eviscerated carcase it appeared at first as if there were two adrenals present on the left side. On further examination however, it was found that this appearance was due to there being two lobes to the left gland. This double-lobed gland was occupying the position in which one would normally expect to find it in the sheep, that it was a short distance from the anteromedial border of the left kidney, and almost in contact with the left renal vein.

The right gland seemed to be quite normal, placed lateral to the vena cava, and lying between the anterior pole of the right kidney, the liver and the vena cava, and about half and inch cranial to the angle of junction of the right renal vein with the vena cava.

Both adrenals were dissected out from perirenal fat and the vessels and nerves which entered each respective gland were severed. The supply to the left appeared to anastomose within the capsule of each glandular lobe as one would expect to find in a normal left gland. In this respect the right gland was normal.

In the case of the left gland, a careful search was made through the surrounding connective tissue for accessory adrenals, or chromaffin tissue. There was none present, though small lymph nodes abounded in the region.

B. Macroscopic Appearance.

Both adrenals were dark red in colour, with pigmentation evident in the capsule. The capsules of both glands however, were smooth and without nodules or undulations of any kind on their surface, either of the type said to be commonly present in the horse by Speed and Morris (1946), or the hyperplastic nodules as seen by Weber (1926), in the human gland. It must be remembered that the specimen in question was obtained from a young animal which would be unlikely to have suffered the stresses which the above authors infer may be the causes of cortical hyperplasia.

(*a*) *Right Gland.*

This adrenal was bean-shaped, being thickest and widest in its middle third, where it was closely applied to the vena cava.

(*b*) *Left Gland.*

The left adrenal appeared to be divided, by a septum arising from the capsule, into two or more or less equal, rounded portions (Fig. II). These lobes were about the size of peas. They were closely in contact with one another, and the long axis of the combined lobes was approximately at right angles to a sagittal plane taken through their junction.

The measurements, weights and displacements of the two glands were taken, and tabulated as follows:—

	Length	*Breadth*	*Weight*	*Displacement*
Right	25.5 mm.	10.9 mm.	2.6 G.	2.45 ccs.
Left	5.7 mm.	9.5 mm.	0.8 G.	0.65 ccs.

In the case of the left gland, the breadth expressed above represents that of the combined lobes. The medial lobe was 4.5 mms. wide; the lateral 5.0 mms. wide, while the length of 5.7 mms. was measured across the junction of the lobes.

The animal was a castrated Half-bred lamb nine months of age, and its dead-weight was 74 lbs. The carcase was passed as fit for consumption by the local meat inspector, but apart from this, no observations could be made as to any changes which may have taken place in the organs which had been removed.

It may be seen in the following table of figures collected for the normal adrenals of animals of approximately the same age and class, over a period of several months, that, while the right gland of this animal concurs with the average, there is considerable deviation from the average in the case of the left gland.

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C. Microscopic Appearance.

Unfortunately, neither gland could be photographed *in situ*, or indeed in its entirety, since experience has shown that owing to the critical nature of sheep adrenal fixation, immersion in fixing fluid within a few minutes of the animal's death, together with free-hand sectioning to allow for good penetration, is essential for optimum results.

Both glands were fixed in Susa and blocked in paraffin. Sections were cut at 7 mu and stained with Haemotoxylin and Eosin. The modification of Foot's silver method for reticulum was also used, this having previously been found to give excellent indication of the extent of the zones of the adrenal by demonstrating the distribution of reticular fibres in the respective layers.

Age	Dead Weight	Left		Right		Weight		Displacement	
		Breadth	Length	Breadth	Length	Left	Right	Left	Right
Mths	Lbs.	mms.		mms.		Grms.		ccs.	
7	61	9.0	24.0	9.0	20.0	1.9	2.1	1.8	1.95
7	59	9.0	22.5	11.0	18.0	2.1	2.0	1.95	2.1
8	77	7.5	27.0	11.5	28.0	2.4	2.9	2.6	2.65
8	74	10.6	26.5	10.0	26.5	2.6	2.1	2.5	1.9
9	71	9.0	26.0	8.5	21.0	2.6	2.7	2.8	2.4
9	74	8.5	22.0	10.5	27.0	2.4	2.4	2.2	1.95
10	72	9.0	23.0	9.0	21.0	2.5	2.6	2.1	2.5
10	76	10.0	28.0	11.0	28.6	2.9	2.5	2.6	2.5
11	72	9.5	22.0	9.0	25.0	2.7	2.9	2.55	2.75
11	74	9.5	27.5	12.0	26.0	2.8	2.6	2.75	2.25
9	71	9.2	24.9	10.2	24.5	2.5	2.5	2.4	2.3

(*a*) *Right Gland.*

The appearance of sections agreed entirely with those seen in other sheep adrenals, the cortex being clearly demarcated into Zona Glomerulosa, Zona Fasciculata, and Zona Reticularis. Surrounded by this cortex were coiled columns of medullary cells and their adjacent venous sinuses (Figs. I and III).

It might be added that the presence of a Zona Glomerulosa has been found to be a constant feature in the sheep's adrenal, contrary to the findings of Elias. (1948).

(*b*) *Left Gland.*

On first examination, sections of this adrenal, stained by Foot's method, indicated that the complete structure might well be described as being a "Siamese-Twin" adrenal, in that each lobe had it own individual capsule, the two capsules being united by a small amount of white fibrous tissue.

This description could have been used if the gland had been morphologically normal, but this was not found to be the case when the parenchyma of the lobes

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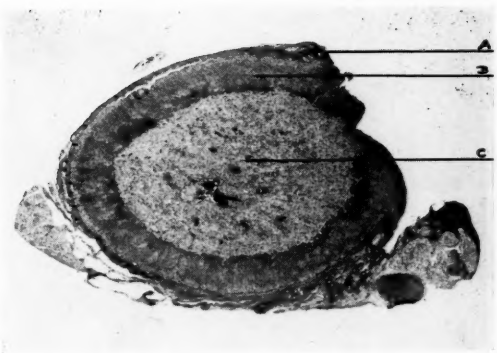

FIG. I—multiplied 10 times

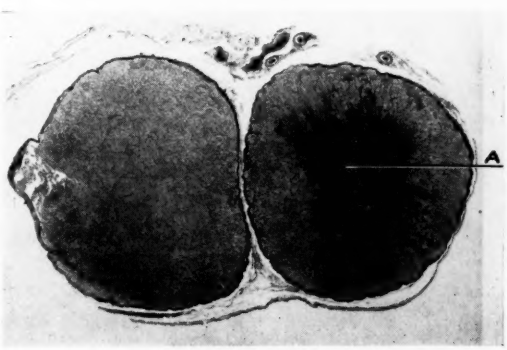

FIG. II—multiplied 8 times

Fig. I

Right Adrenal
A. Capsule
B. Cortex
C. Medulla

Fig. II

Left Adrenal showing double lobation
A. Area of vascularity

Fig. III

Right Adrenal
A. Capsule
B. Zona glomerulosa
C. Zona fasciculata
D. Zona reticularis
E. Medulla

Fig. IV

Left Adrenal
A. Zona fasciculata of Lobe A
B. Zona glomerulosa of Lobe A
C. Capsule of Lobe A
D. Fibrous junction of both lobes
E. Capsule of Lobe B
F. Zona glomerulosa of Lobe B
G. Zona fasciculata of Lobe B

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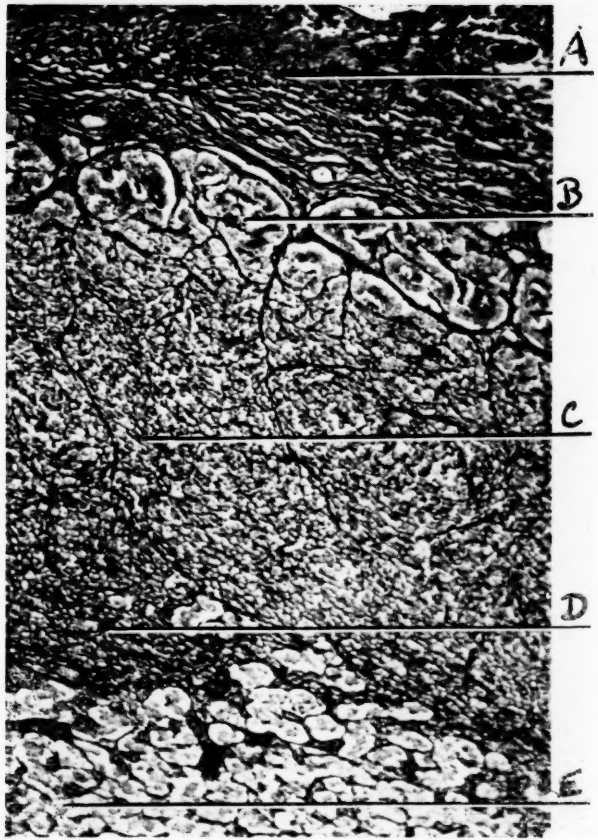

FIG. III—multiplied 115 times

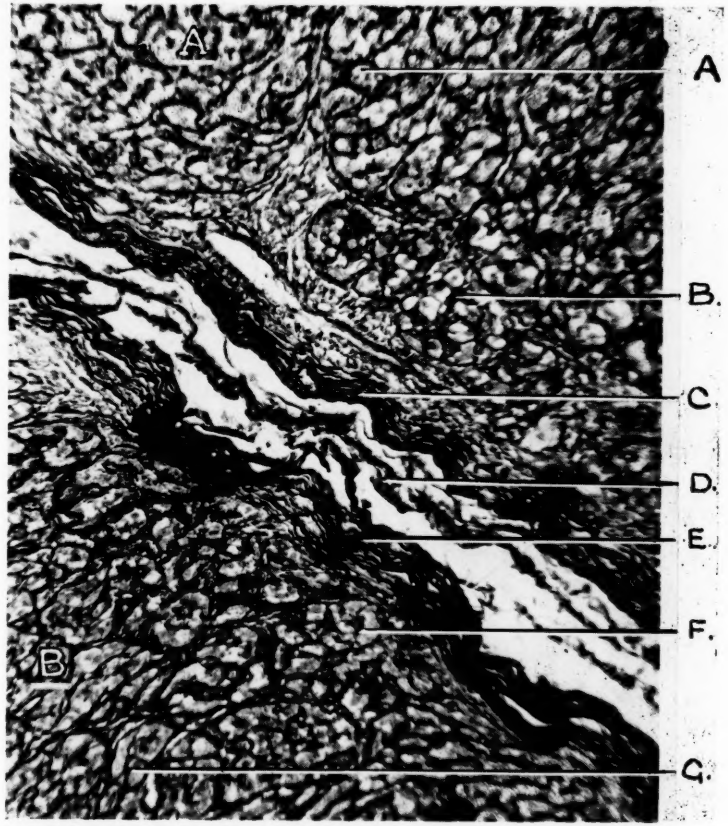

FIG. IV—multiplied 110 times

was examined. In each portion, the Zona Glomerulosa was very distinct; also the Zona Fasciculata. The Zona Reticularis, while appearing to be engorged with blood, was also well demarcated. There was no evidence of medullary cells of any kind present in either lobe (Fig. IV).

Several further sections of the whole structure were cut and stained, but again in none of these was it possible to demonstrate a medulla in either lobe. The remainder of the block was serialised and stained, with the same negative result. Instead of a vacuole or space replacing the absent medulla, the Zona Reticularis had encroached so that reticularis tissue approximated to and interdigitated with reticularis tissue instead of being separated by an intervening medulla.

Conclusions

To explain this adrenal aberration, it is logical to look at the embryological development of the gland. The cortex makes its appearance before the medulla, budding from the cœlomic epithelium situated on either side of the root of the mesentery, medial to the Wolffian bodies, and it is in the mesenchyme here that the first cortical analage becomes apparent.

At the same time, from the neural crest, " sympatho-chromaffin " cells migrate ventro-medially towards these cell masses, and lay down the medullary analage within that of the cortex. Failure of these cells to invade the cortical mass could well account for the abnormality observed, and it might be deduced that some intrinsic factor or stimulus was lacking during embryonic life.

It is well known, that the presence of the adrenal cortex is essential. Mackay and Mackay (1926) refer to compensatory hypertrophy of the cortical tissue of one adrenal after experimental removal of the other. In the case of the adrenal medulla, without which an animal can survive quite comfortably, no hypertrophy need be expected in the medullary substance of one gland if the same tissue of the other is damaged or, as in this case, completely lacking. The present case would seem to confirm this statement.

Acknowledgements

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REFERENCES

Elias, H. (1948): *Amer. J. Vet. Res.*, 9, 173.
Mackay, E. M. and Mackay, L. L. (1926): *J. Exp. Med.*, 43, 395.
Speed, J. and Morris, P. G. D. (1946): *Brit. vet. J.*, 102, 27.
Weber, F. P. (1926): *Brit. J. Dermat.*, 38, 1.

INFUSION AND ANALEPTIC THERAPY
PART 1

By A. M. HARTHOORN, D.V.Sc., F.R.C.V.S.

Their respective values in the resuscitation of dogs from overdosage of pentobarbitone sodium

Introduction

(1) *Value of fluid therapy.*

RESUSCITATION of the patient following overdosage of anæsthetic or narcotic agents has been a formidable problem always, and it has become far more acute since narcotics and anæsthetics that could be given by mouth or by injection have come into general use. In this way a full dose or an overdose can be administered in a matter of seconds and in that way it differs from the far more gradual inhalation method used previously. Once a drug has been administered to a patient by mouth or by injection, it may be difficult or impossible to retrieve. Usually the only loophole left to those wishing to resuscitate the patient is to promote a thorough evacuation or apply physiological antidotes. Usually the former method is applicable only whilst the drug is still in the digestive tract.

For the current anæsthetic and narcotic agents there is no specific chemical antidote once they have entered the circulation*; and physiological antidotes such as drugs that stimulate directly or reflexly the respiration and vasomotor centres are commonly used. In human medicine and surgery, excessively deep narcosis or anæsthesia is combated by the administration of repeated doses of analeptic combined when necessary with other measures such as artificial respiration.

With the complete staff usually available at the average hospital, these measures are practicable, and cases are on record where repeated analeptic administration was continued without a break for as long as 24 hours (Milton and Stennes, 1950). This is possible only where trained orderlies are available to keep a constant watch on the patient, administer injections of analeptic, or apply artificial respiration or mechanical stimulation for long periods of time.

When adequate assistance is not available, the too deeply anæsthetised patient presents a grave problem. Even when spontaneous respiration is present, the continued state of anæsthesia, which may last for two or three days (2½ days in a case personally observed), gives cause for considerable alarm. If no spontaneous respiration is present it very soon becomes questionable whether the continued attention to the one patient, whose ultimate recovery in any case is a matter for conjecture, is justified if in consequence other work is being neglected. One more avenue however is open to the veterinary practitioner faced with a patient who will not respond readily to mechanical stimulation or analeptic therapy. It is mechanical therapy, in which the anæsthetic or narcotic agent is washed out of the blood stream through the agency of physical dilution.

It has been found previously (Harthoorn 1953[1]) that when saline solution is introduced into the blood stream in large amounts it does not remain there but leaves the circulation and enters into the tissues and the serous cavities of the body.

* Since writing, a narcotic agent has been developed and its antidote may be injected I/V. The antidote action would appear to occur in the blood stream.

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It is shown in this paper that when the quantities of fluid are large enough they can cause a dilution of water-soluble anæsthetic agents, sufficient to permit the recovery of the animal.

The introduction of massive infusions is seldom beneficial, in a physiologically normal animal, and still less so in an animal suffering from circulatory failure (Harthoorn 1953[1,3]). It was considered advisable to ascertain the minimum amount of fluid that could be used to obtain results. A quantity of fluid was formulated, which in the majority of cases gave sufficient dilution of the anæsthetic agent in the body to allow of further unaided recovery.

It was found, as will be shown later, that in most of the deeply anæsthetised cases, too deep to respond to analeptic therapy in large and continued doses, fluid infusion of some 20 ccm. per kilogram body weight caused sufficient dilution to enable the patient to breath spontaneously and commence and continue to detoxicate the anæsthetic agent and so make a lasting recovery possible.

The word " lasting " should be stressed. When an apparent recovery has been effected through analeptic therapy the patient may not be out of danger. As quoted cases will show, it may happen that after the subject has been revived, the analeptic agent will be broken down more rapidly than the anæsthetic. In these cases the condition will slowly revert back and become very similar to that exhibited before therapy was applied and the patient may relapse back into coma followed by respiratory failure and death (see Protocols 4, 14 and 15).

As far as could be ascertained, when the fluid that had been introduced to effect dilution of the anæsthetic agent left the blood stream, it took the dissolved substances away with it, or, in the case of Dextraven, maintained them in solution. When resuscitation had been effected by fluid therapy alone, the patient did not relapse. When the stimulating effect was due to a combination of fluid and analeptic therapy the chances of relapse were reduced enormously in comparison to an apparent recovery effected through analeptic therapy alone.

Fluid therapy in combination with analeptic therapy is not entirely an expedient to circumvent the shortage of trained staff in veterinary practice, but it has actual advantage over the use of analeptic therapy alone. It may be applied with advantage even in those cases where apparent recovery has been effected *without* recourse to initial fluid therapy. It is suggested that in these cases the recovery be consolidated by the injection of crystalloid fluid subcutaneously from where it is usually absorbed with reasonable rapidity when there has been no depletion in the oncotic pressure of the plasma proteins (Harthoorn 1953[3]).

On account of the danger associated with the infusion of crystalloid solutions into the blood stream of animals that are suffering from a degree of shock (*vide Supra*) a number of experiments have been performed to show that the resuscitation that can be effected through the infusion of saline solution can also be obtained by the introduction of a solution that remains in the blood stream. Protocols 11, 12 and 16 show that patients suffering from an overdose of nembutal may be revived by the infusion of Dextraven solution, so allowing a possibility of combining the treatment for anæsthetic overdosage with that for hæmorrhage and shock (Harthoorn 1955).

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(2) *Effect of infusions and theories as to action.*

Previous work on materials, which, after infusion into the blood stream, were capable of restoring the blood pressure, revealed that the action of infused fluids works in two principal ways. These two ways take account only of the physiological or physico-physiological actions.

These two actions may be summarised as follows: (1) Where the infused material is composed of molecules or solution of molecules of a size able to exert an oncotic pressure in the blood vessels, and at the same time is not of a nature that is rejected by the body (Harthoorn 1953[1]), the oncotic pressure of the fluid either maintains the infused fluid in the circulatory system, if the blood vessels have not been damaged to too great a degree, or draws inter-cellular or subcutaneous fluid into the blood stream, if the oncotic pressure is greater than that of the plasma. This will result in a rise of the blood pressure either back to normal or approaching normal (Harthoorn 1953[2,3]); or else (especially if isotonic) the fluid will be accommodated in the blood reservoirs. (It has been shown that injections of plasma into healthy animals result in no material increase in the blood pressure or of the circulating blood volume (Harthoorn 1952).)

(2) Where the infused material is composed of molecules that exert no oncotic pressure owing to their small size, such as salt solutions, they will be eliminated from the blood stream if introduced to an amount greater than can be held there by the oncotic pressure of the plasma proteins. In the hypotensive animal they will result in a temporary rise of the blood pressure, followed by an immediate fall (an exception here is the animal that, immediately previously, has been subjected to a rapid hæmorrhage and which is therefore suffering from acute lack of circulatory volume rather than true hypotension). In animals that suffer from circulatory shock this loss of crystalloid fluid from the blood stream will in most cases result in a deterioration of the condition and, after the temporary rise, a further lowering of the blood pressure to below the pre-infusion level.

These two results of infusion of fluid into the circulation may be restated more simply by saying that as to the immediate physico-physiological results of infusions, infusion materials may be divided into those that stay in the blood stream and those that do not.

From the point of simple physical dilution therapy there would be little to choose between the two types of solution mentioned. However, if we include the physiological aspect also, we see that the former has two distinct advantages over the latter.

These two advantages are associated with: (1) The danger of infusing crystalloid solutions into the circulation at times of true hypotension and shock. (2) The advantages in increasing the circulatory volume and therefore also the circulation through the liver at times of even slight hypotension.

The first need be discussed only superficially. It is concerned principally with the difficulty in the diagnosis of true hypotension and the difficulty of differentiation between real anæsthetic overdosage and relative anæsthetic overdosage in the shocked animal, and anæsthetic collapse. The latter, unless treated immediately, will soon result in peripheral stagnation of blood, vascular damage and shock (Harthoorn 1954), while in the case of relative anæsthetic overdosage the treatment

for resuscitation from the anæsthetic must be combined with treatment for shock.

If the infusion of crystalloid solution is administered after vascular damage has commenced in any of the above-mentioned cases, this form of treatment may result in the condition becoming irreversible. Great care must be taken also to try and eliminate cases where there is hypotension or incipient shock due to the original patnological condition. The last is of considerable interest in connection with the effect of a small degree of hypotension on the circulation through the liver and its effect on recuperation from barbiturate anæsthetic. In the case of the infusion experiments described previously (see above) it was found that when suboptimal amounts of barbiturate are given, even long-acting barbiturates, the rate of detoxication is very rapid. In these cases supplementary doses of barbiturate must be administered every hour or so if prolonged anæsthesia is required. If either only a little more anæsthetic is given at the time of induction, or if the original small amount is given during hypotension, the length of anæsthesia is far longer and completely out of proportion to the amount of anæsthetic administered.*

It was shown that similar results could be achieved with the administration of slight excess of anæsthetic or by lowering the blood pressure in other ways. The deepening and lengthening of the anæsthesia in the latter case appeared to be directly due to cerebral anoxia. In both cases the resulting disproportionate prolongation in the length and deepening of anæsthesia may well have been due to vasomotor derangement and consequent lack of circulation through both the brain and liver.

The general circulatory derangement associated with a degree of hypotension or incipient shock resulting in the well-known course of lowered blood volume, lack of venous return, increase in circulation time, stagnant anoxaemia or tissue anoxia, indicated by greatly increased arterio-venous oxygen differences, cerebral anoxia and stupor, is well understood. The degree of derangement of the hepatic circulation remains controversial.

A considerable number of experimental workers are agreed on the finding that, as a result of the lowering of the blood pressure, or in the course of the development of shock, the circulation through the liver is severely curtailed. Blalock and Levy (1937), under the heading " Partition of the Blood Stream " first described clearly how the blood flow through the liver was disproportionately reduced when the blood pressure was lowered. Later, Selkurt, Alexander and Patterson (1947), through studies of the mean portal pressures, found that under the above-mentioned conditions there was a resistance of the blood flow to the liver and a damming back of the blood in the portal circulation.

In this they confirmed similar results by Wiggers, Opdyke and Johnson (1946) and by Engel and co-workers (1944) that under these conditions the oxygen content of the portal blood is disproportionately lowered. The loss of liver function, e.g., the ability of the liver to metabolise glucose, lactate and pyruvate, due to impoverished blood flow is apparently regained after infusion—in this case of blood (Seligman *et al.* 1947).

Thus we may state that the use of substances that remain in the circulation,

* Goldman (1949) records a case of a war casualty who underwent an operation for the removal of a ruptured kidney. The operation lasted 55 minutes. Although anæsthesia was completely adequate a total of only 0.6 grammes of Pentothal was used.

such as plasma substitutes, have distinct advantages over crystalloid solutions in effecting the dilution of anæsthetic in the blood stream, both from the point of view of safety as well as in view of their clear physiological advantages because they increase the blood flow through the brain and the liver in those cases in which the blood pressure may be low. Through their use the hæmolavage will be combined with enhanced detoxication and renewed vasomotor control.

(3) *Types of Solutions used for Infusion Therapy.*

The types of solution that were used as infusion material in the course of these experiments were (*a*) Simple inorganic salt solutions and (*b*) Dextraven solution.* Group (*a*) comprised two different types: firstly a solution of sodium chloride isotonic with the blood, referred to as physiological saline solution in this paper; secondly standard Ringer solution. No special precautions (Rademaker, 1930; Thompson, 1933; Banks, 1934) were employed to eliminate pyrogens.

Harmful effects from the administration of large quantities of fluid to the healthy subject occur readily only when that fluid is hypotonic or plain water (Rowntree, 1923). This does not take into account the presence of actual noxious substances. When excessive amounts of water are administered water intoxication results from the depletion of body salts, especially chloride. Rowntree's results confirmed those of Miller and Williams (1921), who killed rabbits by the administration of some 700 ccm. of water per rectum. Apparently the death of the animals was due to oedema of the brain caused by loss of sodium (Smyth, Deamer and Phatak, 1933), much of the administered fluid leaving the circulation immediately and localising in the inter-cellular spaces. These results correlate with those of Moyer and others (1944), who found that dogs that had recovered from a state of shock would die of oedema of the brain if allowed to drink water *ad libitum*. It appeared that depletion of sodium caused an increase of water in the intra-cellular phase of the tissues.

Isotonic solutions, especially solutions of sodium chloride, seem to have been infused into animals in substantial quantities by a large number of workers without apparent harmful effects. Their results usually confirmed those of Smith and Mendel (1920) and those of Lamson, Oosthuizen and Rosenthal (1923), who found that very large quantities of these types of fluid might be administered intravenously, and that they left the blood stream within a few minutes to enter the tissue spaces or to be excreted into the stomach and intestines. Intra-cellular oedema does not seem to have followed on the administration of isotonic fluids. These results were confirmed by Smith (1925), Kinsman and others (1928) and Collins (1934), who was able to recover a proportion of the injected fluid from the peritoneal cavity. Miller and Poindexter (1938) also recovered a proportion as saliva and vomit.

Cutting, Lands and Larson (1938) carried out large-scale experiments and found that cats died after the infusion of 500 ccm. of saline per kilogram body weight (25 times the amount used in the experiments reported in this paper), although smaller amounts were lethal when the solutions were hypertonic.

When administered in very large quantities, saline solution seems to be harmful, owing to the salt excess (Jones and Eaton 1933), although in the course of later

* Kindly supplied by Benger Laboratories Ltd.

work the removal of the plasma proteins was thought to play a role (Jones, Eaton and White 1934). The reduction of the plasma protein as a result of massive saline infusions was also the result of the experiments of Blalock and Beard (1932).

Other reports on the infusion of saline solution have generally been concerned principally with their beneficial effect in relation to pre- or post-operative dehydration of the body water balance in disease. These have less direct connection with the experiments reported here and therefore will not be discussed. (Reference, however, may be made to the following: Cantaro (1939), Water balance, Oedema and dehydration; Maddock and Coller (1937), Water balance in Surgery; Maddock (1938), Fundamentals in water balance; Coller and Maddock (1940), Water and Electrolyte balance; and Ravdin (1940), Symposium on fluid and electrolyte needs.)

Many solutions of salts other than sodium chloride solution, have been tried experimentally, and some appear to have certain advantages, such as the correction of acidosis. Apart from the possible advantage in the utilisation of fluids that more closely resemble the composition of the blood, such as Ringer solution, there appears to be no advantage in using other solutions than sodium chloride for the purposes of dilution therapy. Some of the various solutions that have been suggested at times such as Sodium lactate by Hartman and Senn (1932) and Fox (1944) and Sodium bicarbonate by Wiggers and Ingraham (1946) and Ammonium chloride by Zintle and others (1943) need not be reviewed here, for the experiments recorded in this paper were concerned with simple dilution therapy rather than with the possibility of finding a chemical antidote, and their scope did not allow of the trial of crystalloid solutions other than physiological saline and Ringer solutions. Dextrose solution was considered unsuitable because although it may be isotonic with blood at the time of infusion, the Dextrose becomes oxidised when assimilated by the body and pure water remains. When Dextrose solution is administered in quantity, therefore, water intoxication might result.

Dextraven solution was used to represent that class of fluids which appear to exert a definite colloid osmotic, or oncotic pressure after they have been introduced into the circulation. It has been incorporated into these experiments for the following reasons: (*a*) It would give a better indication whether the results of infusion therapy on anæsthetic overdosage were due to simple dilution rather than a washing out of the agent into the tissue spaces and serous cavities; (*b*) It would answer the question whether such solutions would be equally effective; an important question, in view of the fact that in certain cases of circulatory imbalance it is not desirable to introduce crystalloid solution into the blood stream and, in cases where the diagnosis of the circulatory conditions is uncertain, it may be imprudent to do so.

The results obtained from the infusion of Dextraven by an number of workers are reviewed elsewhere (Harthoorn 1956).

(4) *Review on Cardiozol* and Picrotoxin.*

The standard method for counteracting the effects of anæsthesia is the administration of analeptics; and in the work that follows it was necessary to compare the effect of fluid therapy and hæmo-dilution with such a standard procedure. A

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short review of reports on the two analeptics used is given below to explain the reason for their choice.

In their report to the Council on Pharmacy and Chemistry, Eckenhoff and others (1949) define ANALEPTICS as follows: "A drug that is capable of stimulating the normal central nervous system and which is used for overcoming a depression of that system." They furthermore classify analeptics as follows:—

(1) Those that have a primary stimulating action on the nervous system and the value of which depends on that action. As examples Picrotoxin, Cardiozol (Metrazol), Nikethamide were given, as well as Strychnine, Caffeine and Atropine (the last three were regarded as obsolete). (2) Drugs the analeptic effect of which is due to reflexes from one source or another. (3) Drugs which stimulate both the central nervous and cardiovascular systems. Examples given are Benzedrine, Dexedrine, etc. (4) Substances which exert a specific stimulant effect on nervous tissue by entering directly into the biochemical reactions on which the excitability of this tissue depends, such as pyruvate and components of the vit. B complex.

The authors state that the most probable explanation of the reciprocal relationship of analeptics and depressants is physiological antagonism. They first of all discuss Picrotoxin and Cardiozol and suggest that, although less powerful in its action than Picrotoxin, Cardiozol has a more potent action on the general nervous system. They cite evidence to suggest that the two might be used together so that the one might act as an adjunct to the other. They state that all available evidence indicates that Nikethamide is definitely inferior to Cardiozol or Picrotoxin.

The sympathomimetic amines of group 3 are suggested as being useful for psychic depressant states rather than narcotic poisoning. Their report is concluded by the statement that Picrotoxin and Cardiozol are the most valuable analeptics obtainable today, with Picrotoxin as the most powerful but also the most dangerous.

Besides that mentioned above, there are very many reports in the medical and experimental literature on the properties and advantages of both Picrotoxin and Cardiozol in their function as analeptics and as physiological antidotes to anæsthesia, especially barbiturate anæsthesia. A comprehensive review has no place here. A few of the more important works on this subject may be mentioned.

Bremer for instance in 1936 found it was obvious that Cardiozol acted on the brain centres. This was confirmed by Schulte, Tainter and Dille (1939), who further narrowed the location of action to the sub-cortical region. Richards and Menaker (1949) found that Cardiozol was more effective in its action on the higher centres than Picrotoxin. A combination of the two has been advocated by Ebes (1939). Bleckwenn and others (1940) advocated the use of Picrotoxin and Cardiozol among other preparations, while Jones, Dooley and Murphy (1950) give preference to the latter in a comparison between the two.

Barlow (1938) reports favourably on the use of Picrotoxin and Cardiozol for barbiturate poisoning in rabbits and prefers them to substances such as ephidrine; and Hjort and others (1938), working with mice, could find no difference in the degree of action between the two. The central pressor action of Cardiozol was described by Hahn, Plester and Rummel in 1950 and its effect on asphyxia in the newborn by Leak (1944, 1947 and 1948) and by Barber and Madden in 1944.

Clinical reports on the use of individual, or a small number of cases of

Cardiozol therapy are reported by Eggleston (1936), Frölich, in veterinary practice, in 1938, as also Gronau (1940) and Kantscheff (1941), likewise, in animal medicine; against Pentothal by Pickrell and Richards (1945) and on dogs against Amytal anæsthesia by Rice and Isenberger (1937); as therapy for dogs under pentothal anæsthesia by Reynolds (1939); and Picrotoxin and Cardiozol for Evipan anæsthesia by Das (1939). Against Nembutal anæsthesia Werner (1938) and Werner and Tatum (1939) claim that in rabbits Picrotoxin is stronger in action than Cardiozol. This was confirmed in 1945 by Goodwin and Marshall, working with mice as their test animals.

A favourable report on the use of Cardiozol, as antagonist to Nembutal in the dog, was written by Morris and Eastman (1936); they cite Fulton and Keller, who apparently obtained similar results with the same products on monkeys. Similar reports have been made by Burn (1939) and Jackson (1934).

It seemed from these and other reports that Picrotoxin and Cardiozol would be suitable representatives of the class of products with analeptic action which one could use to compare the value of analeptic therapy with that of infusion therapy.

Methods

The experiments were conducted along four main lines.

(1) The resuscitation of the anæsthetised animal with Picrotoxin.

(2) The resuscitation of the anæsthetised animal with Cardiozol.

(3) The resuscitation of the anæsthetised animal with physiological saline solution (or other isotonic crystalloid solution).

(4) The resuscitation of the anæsthetised animal using a combination of Hæmolavage and analeptic therapy.

They can be divided further into two main groups: (*a*) Non-survival experiments where the blood pressure was measured. (*b*) Survival experiments where the blood pressure was not measured, where the saline solution or Dextraven used was infused under aseptic precautions and where, except for the controls, the recovered animals were allowed to live indefinitely.

The procedure used in group "*a*" was as follows:

The animal was anæsthetised with pentobarbitone sodium (Nembutal Abbott labs.) until corneal and pedal reflexes were absent.

The carotid artery was cannulated and connected to a mercury manometer.

The trachea was cannulated and prepared for connection with respiratory apparatus. The femoral vein was prepared for injection. The animal would then be left in this condition to recover from the effects of the procedure described above. After the lapse of 15 minutes the blood pressure was measured and, if it was normal, the experiment was continued.

Nembutal was now injected until respiration ceased. By "ceased" is meant that no respiratory movement was made over an observation period of two minutes. After the lapse of the period of two minutes, the tracheal cannula would be connected to the artificial respiration machine and a further respiratory test would be made after a lapse of 15 minutes. Then a further quantity of nembutal, amounting to 20 per cent. of the dose originally needed to produce loss of the pedal and the

corneal reflexes plus that used for producing cessation of the respiration, was injected. Each dose was first biologically standardised for the individual animal. This method was thought to give more reliable results than a calculation of the amount on the basis of body weight. The animal would now be left for a further 15 minutes, after which the respirations were again tested over a two-minute interval. If during this time a respiratory movement was made, the animal would be discarded for the purpose of that experiment.

If no respiratory movement was made the substance under experiment was injected. After the injection of analeptics a further 15 minutes was allowed between the injection and the trial of spontaneous respiration.

Artificial respiration was regulated carefully so as not to over-ventilate the animal. The blood pressure was measured at each respiratory trial.

The measurement of the blood pressure ensured that the animal did not go into a state of undetected circulatory shock as a result of the large injection of the anæsthetic agent, and also that no intense circulatory derangement following on the use of the drugs employed was allowed to go undetected and by its presence give a false impression of the state of anæsthesia.

The measurement of the blood pressure was also one of the criteria whereby judgment could be made of the state of ventilation of the subject.

Survival was assumed to be certain when the subject made continuous improvement over a period of four hours after treatment.

The procedure in the "*b*" group was similar except that the carotid artery and the trachea were not cannulated. The blood pressure was not measured and ventilation was carried out by simple rhythmical pressure on the ribs of one side by an assistant. An endotracheal tube was placed in position in all cases. In these series no blood vessels were prepared for injection other than by depilation and sterilisation of the skin. All injections were made by sterile small bore needles into the radial vein (occasionally into the recurrent tarsal).

All infusions in this group were allowed to proceed slowly, and the amount of 20 c.c. per kilogram body weight was infused over some 30 minutes instead of less than 10 minutes as in group (*a*).

No restricted or arbitrary time was fixed to denote survival, but at the termination of the experiment the animal would be removed from the table and wrapped in a blanket.

It would be counted as having survived only when it was walking and feeding normally on several subsequent days.

The general procedure for the two groups was as follows:

All animals were healthy as determined by behaviour, appetite and simple clinical tests. No selection of animals has taken place, the experimental procedure in question being determined before the subject was obtained. All dogs were kept under observation for a period of at least three days between purchase and utilisation. All dogs were mongrels. These stray dogs were kept in the local pound for several weeks before they could be acquired. The condition of those that survived this time without being claimed by members of the local population usually defied description.

The protocols have been written in the approximate order of enaction. No

protocols of complete experiments have been discarded. Twenty experiments were made and the results of seventeen have been recorded. The remaining three animals reacted as follows: two dogs died of cardiac arrest during the injection of the large amounts of barbiturates. One of the first group, whose carotid cannula became disconnected while temporarily in charge of an assistant, was destroyed by chloroform according to pre-arranged emergency procedure.

No operational procedure, other than the insertion of sterile small bore (intravenous type) needles into a superficial vein, was carried out in the course of the experiment on those animals that were allowed to regain consciousness.

Results

The protocols reporting the results of the experiments can be divided for the purposes of discussion into three groups.

(1) Those in which resuscitation was attempted with analeptic therapy alone.

(2) Those experiments where resuscitation was effected by a combination of analeptic and infusion therapy.

(3) Those experiments where the subject was treated by infusion therapy alone.

The first group (see protocols 4, 14 and 15) were all ineffective ultimately. This is not an indication of ineffectiveness of analeptic therapy. It is, however, an indication of the dangers and difficulties associated with analeptic therapy when this is not continued until complete and lasting recovery has been effected. No. 10 would doubtless have followed the same pattern but before the analeptic had time to become completely ineffective, saline solution was administered, thus ensuring that recovery was maintained.

The second group of experiments may be divided into a further two subdivisions.

(*a*) Those in which the analeptic in question was administered first.

(*b*) Those in which infusion therapy was carried out either primarily or after other forms of therapy and in which an analeptic was administered subsequently.

If the experiments were to be performed again now, after certain facts have emerged clearly from these trials, no attempt would be made to perform any number of experiments in which analeptic would be used as a sequel to infusion therapy. The reason for this is that in the third group of experiments (the last to be performed) it is shown that with the dosages used the infusion, if it is given at physiological speeds, was sufficient in every case to effect recovery without recourse to other forms of therapy. In the first pilot group of experiments it was not realised that, when the infusion is administered at a very rapid rate (e.g., 20 c.c. per kg. body weight in less than ten minutes) the fluid administered will not have time to act. The reason for this is not completely clear, but it is in all likelihood associated with balance of anæsthetic between the tissues and the blood stream. In the last and smaller group of experiments, where the process was carried out to resemble as closely as possible the conditions obtaining in veterinary, rather than laboratory practice, it was found that when the infusion was given over a period of 30 to 40 minutes, sufficient time would have been allowed and the animal would show spontaneous respiration at the end or before the end of the completion of the infusion. It has been assumed from this that had, in the second group, artificial

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respiration been carried out for some 15 or 20 minutes subsequent to the termination of the infusion, the animal would have breathed spontaneously and without other treatment. This is borne out to a large extent by protocols 2, 7, 9 and 17.

The third group of experiments comprises those in which infusion therapy only was used. These protocols (Nos. 2, 7, 11, 12, 13, 16 and 17) show that it is quite possible to effect recovery and survival of the patient using only infusion therapy, even in the high overdosages of anæsthetic as used in these trials. The experiments in which this form of therapy was used in the course of the second group, show that apparently it is quite safe to cease therapy as soon as the animal breathes sufficiently to maintain life. In all cases (protocols 11, 12, 13, 16 and 17) the animal, when removed from the table and left to complete recovery on its own, was able to survive indefinitely without any apparent ill effects from either the overdose of anæsthetic or the subsequent infusion.

Results of only passing interest, such as the number of times convulsions were elicited through analeptic administration, are listed in the Keys to the protocols, I and II, and are mentioned where pertinent or of interest in the Discussion.

(*Part II will appear in the October issue.*)

PLANNED RESTRICTION OF RADIOACTIVE UPTAKE BY ANIMALS

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THE increased uptake of radioactive isotopes and particularly that of strontium-90* by animal bones has aroused concern. While the uptake of radioactive materials by animals is likely to cause damage and economic loss, their transfer to man by milk may give cause for even more concern. Restriction of absorption and of uptake of strontium-90 from food by man is being considered elsewhere (see Lewin, 1957). Methods which are based on physical, chemical and biochemical considerations and on experiments are dealt with here and these are applicable to the restriction of intake and uptake of strontium-90 by animals. There are two complementary approaches :—

(1) Decreased *intake* due to decreased strontium-90 concentration resulting from applied adsorption, ion exchange and precipitation of insoluble salts, and

(2) Decreased *transport* and decreased *uptake* owing to the presence of other ions having similar chemical properties to the strontium ion, e.g., calcium.

The decreased intake is based on a series of in vitro experiments.

The shaking of " insoluble " phosphate powders, such as those of magnesium and calcium, with solution of strontium salts resulted in considerable proportion of the strontium ions being removed from solution. This removal can also be observed on passing solutions of strontium salts through columns packed with the above phosphates. Almost complete removal of strontium can be achieved

* Other radioactive strontium varieties and fission products are also bone-seekers (Hamilton 1947, Brues 1949, Vaughan & Tutt 1953) but the isotope giving rise to much concern is the strontium-90 variety.

depending on the relative proportions of the phosphate powders to the quantity of strontium salt—as well as dilution—in contact and on duration of contact. For example, over 95 per cent of the strontium content can be removed within a minute of shaking about 100 ml. of 0.02 M strontium chloride solutions with 5 gm. of finely divided calcium phosphate. The experimental results are in agreement with the interpretation of a preliminary adsorption followed on prolonged contact by ion exchange and strontium phosphate precipitation. Experiments made on several soil samples having pH values between 5 and 8 from which most of the calcium and magnesium as well as the anions of phosphate, carbonate and sulphate have been removed, and on samples of the same batch to which calcium and magnesium phosphates have been added, show that the latter have much greater capacity for strontium removal. The above phenomena are not limited to " insoluble " phosphates, as calcium and magnesium carbonates and calcium sulphate suspensions remove strontium similarly.

The phenomenon of adsorption is probably general, since these insoluble salts display initial absorption of some soluble calcium salts, but the subsequent precipitation of strontium salts noted is due to the strontium salts being more insoluble than the corresponding calcium salts. While accurate data concerning strontium phosphate are not available, the expectation that strontium phosphate is less soluble than calcium phosphate is in line with other data* and with experimental observations in this laboratory.

The above experiments show that the addition of suitable powders such as calcium phosphate and sulphate would decrease considerably the concentration of strontium (including strontium-90 which is identical chemically with ordinary strontium isotopes) in soil water and consequently decrease the chances of strontium intake into grass and other plant roots. As such plants constitute the staple diet of cattle, reduction in intake of strontium-90 by cattle grazing on pastures, treated with the above powders, should follow. Further details relating to restriction of strontium-90 intake by plant roots will be given later.

Decreased transport and uptake

(a) *Transfer and transport.* Strontium ions (including strontium-90) being chemically similar to calcium will follow the same path using the same enzyme systems. For a given *saturated* enzyme system, the ratio $\frac{\text{concentration of } Ca^{++}}{\text{concentration of } Sr^{++}}$ will increase with increase in concentration of the calcium ions present. This applies to transfer across biological membranes, such as root or gut, and to transport to bone or milk production centres Hence, addition of calcium salts to the soil, by favouring increase in the above ratio, should result in decrease in both intake and subsequent uptake of strontium-90.

Since enzyme systems carrying the calcium and strontium do not distinguish between ordinary strontium isotopes and strontium-90, increase in the ratio $\frac{\text{concentration of non-radioactive strontium}}{\text{concentration of strontium-90}}$ will result in decreased transport of

* For example, at a room temperature, the solubility product of strontium carbonate is ca. 5×10^{-9}, while that of calcium carbonate is ca. 2×10^{-8}: the solubility product of calcium sulphate is ca. 2×10^{-4}, while that strontium sulphate is ca. 3×10^{-7}.

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strontium-90. Consequently, addition of non-radioactive strontium salts to the soil should result in decreased strontium-90 content of plants, and therefore of the animals which graze on them.

(b) *Deposition.* In agreement with Robison's experiments (1923), the deposition of calcium phosphate in bone may be explained in terms of the solubility product principle, though not necessarily in terms of an initial trivalent phosphate deposition. The same pattern of precipitation can reasonably be expected to apply to the precipitation of strontium phosphate, since calcium and strontium are chemically similar. On the basis of this principle the products of the ionic concentrations

$$\{[Ca^{++}]^3[PO_4^{---}]^2\} \text{ and } \{([Sr^{++}_{ord}]_+[Sr^{++}_{90}])^3[PO_4^{---}]^2\}$$

must be limited. Consequently, for a given phosphate concentration, partly controlled by a saturated phosphatase system, each of the quantities $[Ca^{++}]$ and $([Sr^{++}_{ord}]_+[Sr^{++}_{90}])$ in solution must be limited. When the concentration of the calcium ions in solution is—temporarily—greater than the permitted one, precipitation of calcium phosphate follows, thus tending to keep the soluble phosphate concentration at a lower level. This decreases the chances of the ionic product of strontium ions and phosphate ions exceeding the solubility product value of strontium phosphate and its consequent precipitation. The same approach, though modified, applies to the deposition of calcium and strontium as carbonates or to the deposition of a mixed phosphate-carbonate as in the case of apatite which has been taken to represent the pattern of deposition of calcium in bone.

It should be noted, however, that the presence of complexing agents such as citrates or aminoacids and the relative stability constants of their complexes with calcium and strontium may, by controlling the concentration of the non-complexed Ca^{++} or Sr^{++}, play an important, if not decisive, factor as to the proportion of the particular metal in the deposit formed. Qualitatively, increased calcium ion concentration must favour decreased incorporation of strontium—including strontium-90—in biological organisms.

Statistical analysis of cases involving high availability of calcium ions such as cattle or human beings in districts with a high degree of hardness of water, or intake of calcium salts, when added to the food, provided that no associated increased concentration of strontium-90 is present, should reveal a lower strontium-90 content. However, the statistical analysis may be affected by the precipitation of calcium phosphate and strontium phosphate in those portions of the gut where absorption is to take place and where alkaline conditions favour such precipitation. In such cases the advantage of partial precipitation of the strontium phosphate and the presence of calcium phosphate may be counterbalanced by the disadvantage accruing from the decrease in concentration of the calcium originally present.

The possibility of increased concentration of strontium-90 accompanying increased hardness of water should be considered. As is well known, the bicarbonates of calcium and magnesium are responsible for the temporary hardness of water, while the corresponding sulphates and chlorides give rise to

permanent hardness. Rain water which contains strontium-90 and CO_2, on reaching the soil, dissolves some of the magnesium and calcium carbonates present as their bicarbonates. When gypsum deposits are encountered some calcium sulphate is dissolved, but here again a selective precipitation of the much more insoluble strontium sulphate is to be expected. Consequently, natural waters with a high degree of hardness should be considered superior to soft water, because on the whole they must contain less strontium-90 (strontium carbonate is less soluble than calcium carbonate) and because of their high—beneficial—calcium content.

In this connection the parallelism noted between soil calcium deficiency and uptake of strontium-90 by plants and animals (Booker *et al.*, 1957, Libby, 1956a and b) feeding on them should be considered. We consider this parallelism to be only *partial* in that it is also due to associated deficiency in the anions of phosphate, sulphate and carbonate. This is based on the following physical-chemical considerations:—

The metal-aluminium-silicates (or zeolites) of the clay present will tend to remove some, if not much, of the strontium-90 present in the rainfall by an initial adsorption followed by ion-exchange such as

$$M^{++}(\text{Aluminium-silicate})^{--}+Sr^{++}_{90} \rightleftharpoons Sr^{++}_{90}(\text{Aluminum-silicate})^{--}+M^{++}$$

and by precipitation of insoluble sulphate, phosphate or carbonate in the presence of the latter anions. In mountainous districts continuous removal of metal salts of the above anions is bound to result in a smaller uptake of insoluble strontium-90 salts by the soil; consequently, the distribution of the insoluble strontium salts will be less concentrated. That, by itself, should not affect the concentration of strontium-90 in soil water, since at equilibrium the concentration of a sparingly soluble salt in solution is independent of the quantity of solid present. Intake of inorganic salts—and therefore of metal ions—from the soil is associated with the excretion of organic acids, from the root, into it. It is well known that organic acids such as citric, lactic or amino acids increase the calcium solubility by complex formation; it was therefore to be expected that this would take place also in the case of strontium. Indeed, experiments show that the "insoluble" salts of strontium, e.g., phosphate or sulphate, dissolve appreciably in contact with *concentrated* solutions of the salts of such acids. This effect was confirmed by passing dilute solutions of the complexing salts through columns of earth which were freed as far as possible from phosphates, sulphates and carbonates and which contained both calcium and strontium and through columns of earth from the same batch but to which was added magnesium phosphate powder. Though reproducibility varied with the method of mixing the powder and with the type of soil used, qualitatively the presence of phosphate considerably restricted strontium elution. The initial soil uptake of strontium-90 from its solution in rainwater can therefore be partially reversed by re-solution in presence of the above complexing anions, but to a much lower extent than would take place in the presence of phosphate (and by inference also of sulphate and carbonate). Any deficiency of calcium in the soil, resulting in a low $[Ca^{++}]/[Sr^{++}]$ ratio, is now followed by increased strontium-90 incorporation into the plant.

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On this basis, Libby's suggestion (1957)—based *empirically* on the experimental parallelism between soil calcium deficiency and increased strontium-90 content in plants and animals—that the observed conditions could be relieved by fertilisation of the soil with calcium (either calcium nitrate or lime being used) is only a *partial* solution of the problem. A more effectve solution is the addition of such " insoluble " powders as calcium phosphate, calcium sulphate and calcium carbonate (the latter powder being less effective since it dissolves to form soluble bicarbonates which are washed away, and since strontium carbonate is only a little more insoluble than calcium carbonate). As previously stated, the results of the investigation of strontium (and by inference of strontium-90) uptake by several soil samples are in agreement with the pattern suggested here. The usefulness of addition of non-radioactive strontium salts to the soil by decreasing the proportion of strontium-90 to non-radioactive isotopes is probably much more than counterbalanced by the resulting overall decrease in the intake of calcium.

In considering the general strontium-90 chain ending in animals, the first and probably the simplest step would be to *increase* the uptake of strontium-90 by the soil by ensuring a selective high degree of insolubility as well as low competitive ability compared with calcium for entry into the roots of plants. This can be advanced as suggested. Further steps involving restriction of transport and deposition in bone of strontium-90 will be favoured by a higher intake of calcium salts resulting in a higher $[Ca^{++}]/[Sr^{++}]$ ratio. Since dairy products possibly account for about 80 per cent of food intake by humans (Kulp *et al.*, 1957, Report 1954) and even more by very young animals, the possibility of treatment of milk, with high strontium-90 content, may arise. It should be possible to " destrontify " milk by the use of ion-exchange—e.g., calcium-aluminium-silicates—methods. Provided no further production of strontium-90 takes place on a large scale, this may not be necessary with soil treatment and with calcium additions to the diet.

The problem of treating animals already suffering from high strontium-90 content in bone may however arise. It is known that calcium and strontium present in bone are, despite their " insolubility ", in a dynamic equilibrium with the surrounding tissues. Therefore, given sufficient time during which the $[Ca^{++}]/[Sr^{++}]$ ratio is rather high, due to intake of calcium salts, elution of bone-strontium-90 will follow. In cases where rapid removal of strontium-90 is essential, it is suggested that another method be additionally employed, viz., treatment with calcium disodium ethylene diamine tetraacetate (Ca disodium EDTA) (Schwarzenbach and Ackermann, 1948, Sidbury, 1955). The administration of this compound has been found to result in exchange of its calcium for lead initially present in bone, and to lead to increased elution of the lead—and its subsequent excretion by the kidney—without any ill effects. In vitro experiments show that interaction between calcium sodium EDTA and strontium phosphate and carbonate solids result in dissolving of the latter accompanied by the formation of the soluble strontium EDTA analogue. It can be reasonably expected therefore that this will also take place readily in vivo.

The present method of assaying the radiostrontium content of animals is to examine that of their bone ash. Such post-mortem examinations are naturally final. In the case of fowls, however, the laying of eggs with their carbonaceous

shells enables a continuous method of assay. Since the same enzyme system is responsible for calcium carbonate and strontium carbonate deposition in both bone and egg shell, the calcium/strontium ratio in egg shell should be in a definite proportion to that in bone. Consequently, assay of radiostrontium content in fowls subjected to its effects can be followed at intervals by egg shell analysis without resort to post-mortem methods. In view of other pressing research commitments, the author is unable to follow this line of investigation, and it is therefore hoped that it will be taken up elsewhere.

Summary

Methods of decreasing radiostrontium intake and uptake by animals are advanced on the basis of physical chemical and biochemical considerations and experimental work carried out by the author. The *empirical* findings that cattle grazing on calcium deficient pastures have a higher radiostrontium content in bone are explained as being due not only to low calcium/strontium concentration ratios but also to a deficiency in anions which result in strontium salts which are more insoluble than the corresponding calcium ones, viz., phosphate, sulphate and carbonate. Lines of future research are advocated.

REFERENCES

Hamilton, J. G. (1947): *Radiology, 49,* 325.
Brues, A. M. (1949): *J. clin. Invest., 28,* 1286.
Vaughan, J. and Tutt, M. (1953): *Lancet,* 2. 856.
Lewin, S. (preliminary communication) (1957): *Lancet,* p. 1251.
Robison, R. (1923): *Biochem, J., 17,* 286.
Booker, D. V., Bryant, F. J., Chamberlain, A. C., Morgan, A. and Spicer, G. S.: "Radiostrontium and Radiocaesium Measurements in Biological Materials to December, 1956." Atomic Energy Research Establishment (1957), London.
Libby, W. F. (1956a): *Science, 123,* 657.
Libby, W. F. (1956b): *Proc. Natl. Acad. Sci. U.S., 42,* 365.
Libby, W. F., in a paper read to the American Physical Society, as quoted in the *Lancet* (1957), May 11.
Kulp, J. L., Eckelmann, W. R. and Schubert, A. R. (1957): *Science, 125,* 219.
"Agricultural Statistics." U.S. Dept. of Agriculture, 1954 Annual Report, Government Printing Office, Washington D.C.
Schwarzenbach, G. and Ackermann, H. (1948): *Helv. Chim. Acta, 31,* 1029.
Sidbury, J. B. (1955): *Amer. J. Med., 18,* 932.

RECENT OBSERVATIONS ON RINDERPEST IMMUNISATION AND VACCINES IN NORTHERN NIGERIA

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Introduction

It is well known that certain breeds of indigenous African cattle cannot be safely immunised with goat-adapted rinderpest virus, however favourable the circumstances. In East Africa this applies particularly to the Ankole of Uganda and part of Tanganyika.

In West Africa caprinized virus causes a very heavy mortality if used on the humpless, Shorthorn-type cattle, known generally as Muturu and N'dama. Thorne (1950), for example, recorded the loss of all of 12 animals of Keteku type, but these admittedly showed a high infection rate with sub-clinical trypanosomiasis.

The existence of such hypersusceptible cattle has called for more highly attenuated vaccine strains and, amongst these, lapinized virus has been used with considerable success on many parts of the continent (Brotherston (1951 a, b), Piercy (1953) and others in East Africa, Simpson (1954) in the Gold Coast, Fulton (1950, 1951) in Gambia, Birkett (1953) in Sierra Leone.

More recently Mornet *et al.* (1954) and Illartein and Guerret (1954) have described their studies preparatory to the widespread use of lapinized virus for hypersusceptible (N'dama) cattle in various French West African territorities, and Scott (1955) reported the extensive use of "wet" vaccine in the successful suppression of a widespread rinderpest outbreak in the north-eastern part of the Belgian Congo. In the latter instance well over 300,000 Shorthorn-Zebu, Sanga (Bahima) and Zebu-Sanga types of cattle were involved, and previous experience had shown goat-adapted virus to be too virulent.

In Nigeria, caprinized virus, on occasion, produces such severe reactions, or even death, in the predominant local (Fulani) zebus that it cannot be used with equanimity. Such occurrences may develop:—

(*a*) With young animals below 12-18 months of age at any time of the year; the common result is that only stock over this age are inoculated and herds are left with a considerable nucleus of susceptible individuals. Even where immunisation campaigns have been energetically pursued, the level of herd immunity is not, therefore, adequate to suppress completely natural outbreaks of the disease.

(*b*) With susceptible stock of any age during periods of adverse environmental conditions, particularly during the long rainy season and towards the end of the severe dry seasons; this is one reason why inoculations are reduced or even entirely suspended for several months of each year.

(*c*) Where there is a high incidence of sub-clinical trypanosomiasis or other disease which may "flare-up" following reaction to goat virus. This includes considerable areas of Nigeria.

(*d*) In animals being moved on the hoof, along trade routes, for slaughter in the south of the country; it is important that they should not be held up for long periods whilst recovering from severe reactions, and they should not lose condition seriously. Inactivated vaccines are unsuitable because of the time necessary to establish a sound immunity (10-14 days) and because of the cost.

(*e*) In the British Cameroons where there are several hundred thousand Fulani cattle which have had no known contact with rinderpest for several years; it is probable that the use of goat-adapted virus, to immunise them in the event of rinderpest outbreaks, would result in many severe reactions and a considerable mortality.

(*f*) On Government Stock Farms where animals maintained in relative isolation, with good husbandry practised, appear to be highly susceptible to caprinized virus. There is, in some quarters, an impression that Fulani-owned stock now show more severe reactions than they did in the early years of widespread immunisation by goat virus.

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These considerations, whilst in no sense intended to detract from the very important contribution of caprinized virus-vaccine to rinderpest control in West Africa, do, nevertheless, show that it is not to be regarded as the only, or necessarily the best, product for use in Nigerian zebu cattle. Brotherston (1951a) stated that, under adverse conditions, the mortality amongst indigenous East African cattle may reach 20 per cent.

Further attenuation of the strain by goat passage is hardly to be envisaged. Daubney (1948) stated that little or no reduction in pathogenicity for Kenyan cattle took place between the 250th and 400th goat passages. This appears to be true also up to the 650th-700th passage level. A similar position exists with lapinized virus; Nakamura and Miyamoto (1953) reported no appreciable reduction in the pathogenicity of their strain III for Korean cattle between the 350th and over 1,000 rabbit passages. At over 800 passages this virus still caused 33 per cent *mortality* in Japanese black cattle (Fukusho *et al.*, 1953). Both viruses can, therefore, be regarded as "fixed" in virulence for cattle, the severity of reaction reflecting differences in individual susceptibility.

Our experiences with Northern Nigerian zebus are given under three main headings:—

1. The pathogenicity, under laboratory conditions, of caprinized virus for cattle bred by and recently purchased from Fulani owners.
2. Laboratory observations on the use of lapinized virus in similar cattle with particular reference to the standardisation and dose of vaccine.
3. Small field trials to establish the efficacy of lyophilised virus-vaccine under local conditions.

It has been shown that the prevalent belief (Mettam 1951, Gambles 1952, Thorne 1953) in the unreliability of lapinized vaccine for local zebu cattle is unfounded and that the numerous past failures were probably due to deficient standardisation and inadequate dosage, these being due, in large measure, to difficulties in the supply of rabbits. It has also been demonstrated that the level of rinderpest susceptibility of these animals is probably at least as high as that usually recorded for low-grade cattle in Kenya.

Observations on the Pathogenicity of Goat-Adapted Virus for Nigerian Zebus

During the 12 months ending September 30, 1955, records were accummulated on the effect of caprinized virus, at various levels of dosage, on 145 locally purchased cattle between the ages of 1½ and 3½ years. They were all males, with the exception of one animal; a large number had received anthelmintic treatment. Many were used in the routine titration of vaccine batches and in such cases the individual dose of virus has been estimated from the results of the titration as a whole. None of these animals was challenged with bovine virus or treated in any way to control symptoms. The immunity challenge was made with about 1,000 M.I.D.s of dried goat virus.

The incubation period was calculated from daily temperature records, all the cattle being housed throughout the observation period.

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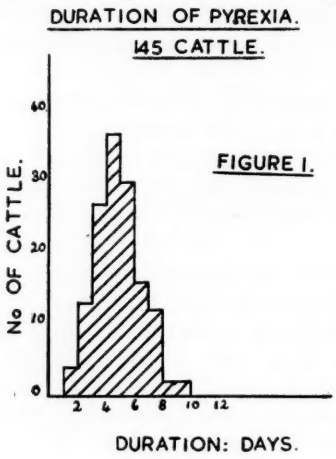
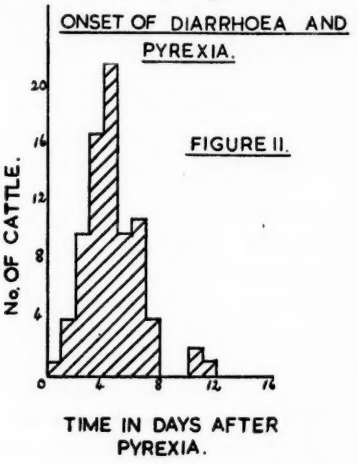
Duration of Temperature Reaction

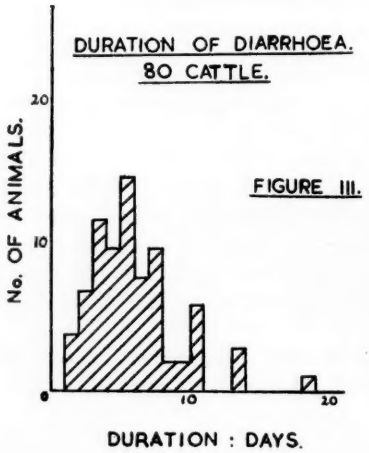
Using the same critical figure, calculations are shown in Table 1 for the mean duration of the pyrexia in groups at three dosage levels, no allowance being made for any secondary peak once the temperature had fallen below 103° F. In two-thirds of the animals the duration exceeded four days, there being, as expected, no appreciable difference between high and low dosage levels. The overall range was two to ten days, individuals being distributed as shown in the histogram (Fig. 1).

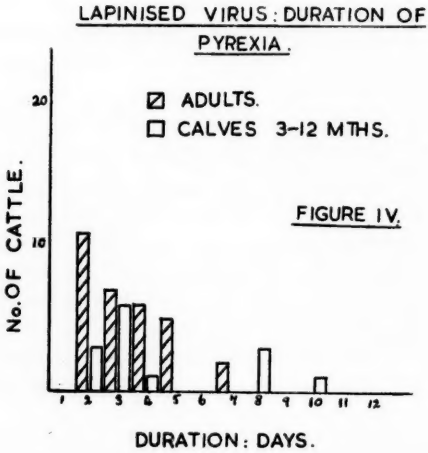
The Occurrence of Diarrhœa

This clinical sign was looked for daily in 114 cattle at the time of taking the temperature. Its duration is difficult to delimit accurately, but has been taken as the period of two or more days when the fæces were completely unformed and liquid. In some cases there was a typical foul smell and streaks of mucus and/or blood. The histogram (Fig. II) shows the time of onset in relation to the first day of pyrexia; Fig. III shows the approximate duration of this sign. Cattle were









housed until diarrhœa had ceased in order to restrict mortality. They were fed on fresh-cut grass or coarse hay, with a supplement of ground-nut cake or cotton-seed.

Eighty out of the 114 animals (70 per cent) showed diarrhœa, which in about two-thirds of them lasted four to eight days; the onset was most frequently on the third to seventh days after the pyrexia.

TABLE I

Incubation Period and Pyrexia in æebu Cattle given Goat Adapted Rinderpest Virus.

Dose of Virus * (Cattle M.I.D.)	No. of Animals Inoculated	Mean Incubation Period (days)	Range in Incubation Period	Mean Duration of Temp. Reaction (days)
10	66	4.96	4-7	5.56
10 -100	33	4.27	2-6	4.33
500 -1000	46	3.06	2-4	5.67

The critical morning temperature was taken as 103°F ; animals with an incubation period of 2 days were often those with a high mean temperature before the inoculation. As the field dose of virus is fixed in Nigeria at an arbitrary 2mg. of satisfactory dried material, containing about 20-40 cattle M.I.D^{50}. it will be seen from Table I that the incubation period may occasionally extend to 6, but is usually about 4 days.

* N.B.—The virus was at its 651st to 653rd goat passage.

Mortality

Deaths occuying within one month of inoculation, in animals which showed a characteristic temperature reaction with other signs of caprinized virus infection, were assumed to have been primarily due to the "immunisation". Table II shows the number of animals dying or killed *in extremis* and the totals inoculated in each month. The data are presented in this way to show that mortality was not limited to one season of the year. A common field observation is that mortality due to goat-adapted virus is higher during the wet season; at Vom this extends from April to September and deaths, in this six months period of 1955, numbered 10 out of 101 animals, i.e., 10 per cent. During the dry season there were nine deaths out of 44 or 20.4 per cent. Overall mortality was 13.1 per cent. Table III shows the time of death in relation to the inoculation date. The third week after inoculation seemed to be most important; in the field, deaths as late as the 5th-6th

TABLE II

Monthly Death Rate in Animals given Caprinized Rinder pest Virus

Month	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Totals
No. Inoculated	4	24	-	-	25	29	27	30	-	9	13	4	165
No. Dying	-	3	-	-	2	3	2	3	-	2	3	1	19

weeks have not been infrequent. The figures presented show that any estimate of the mortality due to goat-adapted virus must be based on an observation period of at least a month; this is very difficult to ensure in Nigeria where herds are dispersed rapidly, as soon as the owners can obtain permission to leave the area of the inoculation camp.

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Animals dying or destroyed *in extremis* commonly showed marked loss of condition and dehydration. Some degree of enteritis of the cæcum and upper colon was usually observed, but the intensity was variable and there were often blackened ulcerated areas at the pyloric end of the abomasum. Mouth lesions of rinderpest were never encountered. Emphysema of the anterior lobes of the

(5)

TABLE III.

DETAILS OF EIGHTEEN ANIMALS WHICH DIED AFTER INOCULATION WITH GOAT ADAPTED VIRUS.

Time of death (days post-inoculation)	Age * (Years)	Dose of Virus (Cattle M.I.D.)	Month of Inoculation	Main Post-mortem Findings
6	-	10 - 100	August	Intense gastroenteritis
7	1½ - 2	500 - 1000	July	Acute haemorrhagic enteritis
10	2	10	December	Diffuse gastroenteritis, pleursy and pneumonia.
13	1½ - 2	500 - 1000	June	Heavy hookworm infestation. Diffuse gastroenteritis Portal cirrhosis
13	2½ - 3	10	August	Portal cirrhosis. Gastroenteritis
15	1½ - 2	10	May	Enteritis
15	1½ - 2	500 - 1000	November	Diffuse gastroenteritis Congestion of lungs
16	1½	10 - 100	November	Hookworm infestation, enteritis
17	1½ - 2	10	May	Enteritis, pneumonia, pleurisy
18	1½ - 2	10	October	Gastritis.
19	1½	10	August	Congestion, emphysema of lungs
19	1½	10	November	Diffuse gastroenteritis
19	1½ - 2	500 - 1000	February	Emphysema of lungs Pharyngitis
20	2	10	February	-
21	1½ - 2	10	October	Gastroenteritis, pulmonary emphysema
25	1½ - 2	10	July	Diffuse gastroenteritis: coccidiosis.
25	3	10	June	Pneumonia, pleurisy (haemolytic coccobacillus)
27	1½ - 2	10	June	Diffuse gastroenteritis emphysema of lungs

* this is an estimate based on permanent incisors and horn-growth.

lung was frequent in animals dying after a prolonged illness; three of 19 cases showed extensive consolidation of the anterior lobes of the lung with fibrinous pleurisy. This condition appeared to be caused in some cases by a hæmolytic coccobacillus; no extensive search has been made for coccidia, but they may sometimes play a part in the development of gut lesions (Lee, 1955). We have

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recently shown that some deaths in the field are due, in part, to *Salmonella dublin* infection. Hookworms may be of importance at some seasons (see Mettam, 1944). All the deaths observed could be attributed primarily to the goat-adapted virus. Table III fails to show any apparent relationship between dose of virus and death rate, but we gained an impression that younger poorly-grown animals were more severely affected; the latter is also a common field experience.

Discussion

Daubney (1948) stated that by the 250th goat passage, Kabete "O" strain rinderpest virus could be relied on to produce an overall mortality of less than 2 per cent in East African Zebus. The mortality rate in Egyptian cattle inoculated with a substrain derived from Nigeria was about 0.25 per cent, and these cattle were presumably of Bos taurus type. Mettam (1943), using the present strain, described the average mortality in "many thousands" of cattle in Nigeria as "well under 1 per cent". Lindley (1952) recorded on overall mortality of 1.59 per cent in about 3,000 cattle given dried goat virus in the Kano Province of Northern Nigeria. Cattle were observed for 30 days following inoculation and the conditions were favourable. He, nevertheless, found that mortality in the animals of individual herds could approach 20 per cent.

So far as zebu cattle purchased today in this part of Nigeria (Plateau Province) are concerned, it is quite clear that goat-adapted rinderpest virus of more than 650 passages had considerable pathogenicity, for those of 18-24 months of age. This pathogenicity was exhibited at the laboratory in spite of the fact that animals were housed to protect them from inclement weather and they were well fed with protein supplements. A mortality of over 10 per cent in these circumstances, quite apart from the considerable loss of condition which is observed in those which recover, would indicate that the generally optimistic field reports might be somewhat misleading as to the true mortality in Northern Nigeria.

Efforts have been made, at various times, to incriminate ancillary factors such as coccidiosis as the cause of post-vaccination mortality; any vaccine-virus which activates these factors is in itself to be avoided. It matters little what the immediate cause of death may be, the fact is that a considerable mortality may follow in favourable conditions, within one month of inoculation. Some deaths may be avoided by treatment with sulphonamides, astringents or antrycide, but this is costly and out of proportion to that of the vaccine, which is a fraction of a penny per dose.

The evidence presented on the susceptibility of Fulani (zebu) cattle to goat-adapted rinderpest virus is supported by observations, to be reported later in this paper, on their very high susceptibility to contact infection with the old laboratory strain of bovine virus, known as Kabete "O" (Daubney, 1948). Evidence will also be put forward that these Northern Nigerian zebu cattle show more frequent and higher temperature reactions to lapinised rinderpest virus than do relatively high-grade cattle in East Africa.

Use of Lapinised Virus in Zebu Cattle: Vaccine Production

Lyophilised vaccine was produced according to the technique described by Brotherston (1951a). The mesenteric gland: spleen: defibrinated blood suspen-

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sion (1/4 w/v) was dispensed in 1.5 ccs. quantities into 5 ccs. ampoules which were freeze-dried on the primary and secondary sides of an Edwards 3 PS machine. Scott and Macleod (1955) have estimated the minimum safe dose of virus for vaccine-producer rabbits as 1,000—I.D.50. We have avoided anxiety on this score by inoculating one or two rabbits with "seed-bank" ampoule material and using their tissues fresh, when they react, as a seed inoculum. A 10^{-2} suspension will contain at least 10^4 I.D.50/ml., and we find that the vaccine rabbits react consistently at about 24 hours and their tissues are harvested on the second day following inoculation. Each ampoule contained an original 0.35G of wet tissues and 1.4 ml. blood (approximately); after drying, the weight of dried material was estimated to be 0.25G, this quantity being issued as equivalent to 125 cattle doses. On an average each rabbit used yielded eight ampoules or 1,000 doses of vaccine. The virus was at its 835th rabbit passage.

Titrations of Vaccine in Rabbits

We commonly pooled the contents of two or three ampoules to limit variations and the primary dilution of 1/25 to 1/50 was centrifuged at 2,000 r.p.m. for 10 minutes in an angle centrifuge. The supernatant was used for further dilutions made in an isotonic phosphate buffer, pH 7.4, prepared as described by Weller *et al.* (1952) and chilled before use. Inoculations were made within 30 minutes, the dilutions being maintained in a bath of iced water.

A shortage of rabbits allowed the use only of sufficient animals to calculate an M.I.D. One ml. of four dilutions from 1/50,000 to 1/400,000 were each inoculated intravenously into two animals. The temperature of these rabbits were recorded three times daily for seven days, and reactions were confirmed by post-mortem lesions. Cultural examinations for Salmonella were made as such infections are not uncommon in our stock of rabbits and the close resemblance of lesions in the two diseases is well known (Nakamura and Fukusho, 1939). No rabbits showing a natural resistance to the virus have been encountered.

Satisfactory vaccine batches were those which produced infection in both rabbits inoculated with the 1/200,000 dilution. Using this method of testing no fall in potency was detected in the batches used after six to eight months' storage at $-20°$ C (we are well aware of the limitations of the M.I.D. as a measure of virus content). This result is in accord with the more extensive data of Purchase *et al.* (1953) and Piercy (1953). It is unfortunate that none of these authors made determinations of virus content in terms of either rabbit or cattle I.D.50; neither is it clear that the initial end-point was found in many instances. Ishii and Takamoto (1953) recorded a slight fall in potency of lyophilised virus after storage for 40 days at $-15°$ to $-20°$ C.

Titrations of Vaccine in Cattle

Three titrations were made in local Fulani-bred cattle, utilising 20 animals in each instance. All were males, between the ages of $1\frac{1}{2}$-$3\frac{1}{2}$ years and in moderately good condition.

In two of the three experiments (Nos. 1 and 2) cattle were housed from seven days before inoculation to the end of the observation period. Daily temperature records (before 8 a.m.) were made. In the third experiment the cattle were not

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housed and were subjected to heavy rain almost daily, this often depressing their morning temperatures to below "normal" limits. In the first trial two or four controls proved to be immune and, as this proportion was far above the 5 per cent or so we have commonly found in cattle of the same source, the result has been included, though open to suspicion. In the two subsequent trials the numbers of the cattle were placed at random into five groups of four, treatments also being assigned at random; in both instances no immune controls were encountered. Dilutions of vaccine were inoculated in 1 ml. dosage subcutaneously.

The challenge for immunity was made on the 11th-31st days, and in experiments 1 and 2 this consisted of the inoculation of 0.1G dried caprinized virus, at least equivalent to 500-1,000 bovine M.I.D.s. A preliminary trial was made to confirm that the immunity engendered in these cattle by lapinised virus was as effective against fully virulent bovine virus as against goat-adapted strains. The result is given in Table IV below:—

TABLE IV

Trial of Lapinised Rinderpest virus in cattle which were then challenged by Goat adapted Virus or Bovine Virus.

adapted Virus or Bovine Virus.

No. of Cattle	Dose of Lapinised Virus (rabbit M.I.D^{50}.)	Challenge Virus	Day of Challenge	No. Immune
4	800 (Approx.)	Caprinised	11	4/4
4	800 (Approx.)	Bovine *	19	3/3 †
2	Nil (Controls)	Bovine	-	0/2
4	Nil (Controls)	Caprinised	-	0/4

* One animal died of blackquarter on the day of challenge.
† The Bovine virus was a locally isolated strain of high virulence.

In the 3rd titration (Expt. 3) a 20 per cent spleen suspension from a bovine animal infected with the Kabete "O" strain, received by courtesy of the Chief

TABLE V

Titrations of Lapinised Rinderpest Vaccine in Cattle

Experiment No.	Dose of Virus (Rabbit M.I.D^{50}.)	No. of Cattle	Day of Challenge	Challenge Virus	No. of Cattle Immune	Estimate of I.D.50.
1	4000	4	14	Caprinised	4/4	
	800	4	14	"	4/4	
	80	4	18	"	3/4	
	8	4	18	"	2/4	15 M.I.D^{50}.
	Nil (Controls)	4		"	2/4	
2	800	4	14	Caprinised	4/4	
	160	4	14	"	3/4	
	32	4	14	"	2/4	
	68	4	14	"	0/4	110 M.I.D^{50}.
	Nil (Controls)	4		"	0/4	
3	4000	4	17	Bovine Kabete "O"	4/4	
	800	4	17		4/4	
	160	4	17(3)31(1)		3/4	
	32	4	17		4/4	32 M.I.D.
	Nil	4			0/4	

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Veterinary Research Officer, Kabete, Kenya, was employed in 5 ml. dosage; this was probably equivalent to at least 10,000 cattle I.D.50.

The results of the three titrations are given in Table V. It was assumed that immune animals had been infected by lapinized virus which had proliferated in their tissues; inoculated animals may be resistant as early as the 3rd day following inoculation (Brotherston, 1951a), due to the interference phenomenon. It was considered that resistance at the 10th day and subsequently implied a true immunity.

Doses in the region of 800 rabbit M.I.D.s produced consistent immunisation; with 80-160, however, 25 per cent of the animals inoculated were still susceptible.

TABLE VI

Infections of Fulani Cattle with the Kabete " O " Strain of Bovine Rinderpest Virus

Ox No.	Method of Infection	Incubation Period (days)	Days with Diarrhoea	Result
408	S/c Inoculation	2	-	Killed 4th day
644	"	3†	4	Died 6th day
622	"	3	-	Killed 4th day
621	"	3	5-14	Killed very weak 14th day
602	"	3	5,6	Died 6th day
603	"	2	5,6,7	Destroyed in extremis 8th day
560	"	2	5,6,7	
490	Contact	Not more than 5	7 - 11	Recovered
628	"	6*	8,9	Died 9th day
1000	"	5*	-	Killed 7th day

† Course almost afebrile.
* Calculated from onset of fever in donor animal.

Estimates of the I.D.50 according to the method of Reed and Muench (1938) are given in the final column, but with groups of four animals such figures can only approximations. The M.I.D. for cattle appeared to be less than ten rabbit M.I.D.s but more than six.

TABLE VII

Results of Inoculations with Lapinised Virus in Adult Zebu Cattle

Dose of Lapinised Virus (Rabbit M.I.D^{50})	No. of Cattle	No. Immune on Challenge	% Immune
c 4000 (10mg.)	45	45	100
c 800 (2mg.)	45	43	95
Nil (Controls)	10	4	40

Objections may be made to the use of goat-adapted virus for challenge purposes, but this virus produces reactions of some severity in local cattle; it is non-contagious and it can be used in field trials with zebu cattle, where bovine virus would be hazardous.

The reaction of Northern Nigerian zebu cattle to the Kabete " O " strain of

bovine rinderpest virus is given in Table VI. It is a rapid and violent one and the infection produced is highly contagious. The latter is of some note in view of previous observations (Brotherston, 1951b, Plowright, 1952) which indicated that the same strain is of low invasiveness for Kenya grade cattle subjected to contact exposure. In Nigeria, as also in Kenya, this strain did not produce mouth lesions.

TABLE VIII

Result of Inoculations with Lapinised Virus in Young Zebu Cattle

Dose of Lapinised Virus(Rabbit M.I.D°. Y	Age of Cattle	No. Inoculated Y	No. Immune	% Immune
o 800 { 2mg. / o 800 { 2mg.	1 Year / 3-6 Months	5 / 18	5 / 17	100 / 94
Totals	——	23	22	96

Field Experiments With Lapinized Virus-Vaccine

These trials were made because the vaccine had become discredited in Nigeria for grade animals or zebus on Government Farms and for cattle of the hyper-susceptible breeds (Maturu and N'Dama) in the "Middle Belt" and the South of the country. Its extensive use in Fulani-owned Zebus or trade cattle had not been envisaged.

A preliminary test was made in 20 half-bred Zebu-Friesian cattle, aged 9-12 months at the Vom Livestock Centre. Such cattle, as previously mentioned, cannot safely be inoculated with goat-adapted virus only. Half were given 2 mg. and half 10 mg. of dried virus from a batch of low titre (M.I.D. 10-5G.). Hence individuals received 200 or 1,000 rabbit M.I.D.s. All were challenged at 14 or 28 days with about 500 cattle M.I.D.s of dried goat virus and all were immune.

TABLE IX

Temperature Reactions of Zebu Cattle to Lapinised Virus

Age of Cattle	No Immunised	Dose of Virus (Rabbit M.I.D°)	No.Showing Reaction	% of Total	Mean Incubation Period (Days)	Mean Duration of Pyrexia (Days)	Range (Days)
Above 1½ Years	68	c 800	19	28.2	6.0	3.7	2 - 7
Above 1½ Years	45	c 4000	12	26.6	5.7	3.0	2 - 7
3 - 12 Months	22	c 800	14	63.6	5.0	4.4	2 - 10

A larger trial was made in 100 adult Fulani-owned Zebus at a station about 40 miles from the laboratory. Temperatures were taken every morning from the day of inoculation to seven days after challenge with about 800 cattle M.I.D.s of dried goat virus. Details are given in Table VII.

The animals were of different ages and the "control" group included an undue proportion of older cows which were afterwards said to have been immunised previously; 92 per cent of the cattle were about two to four years old. Nevertheless, it was possible to ensure the immunity of 95 per cent of Zebu stock

with a (2 mg.) of high potency vaccine. This is a very serviceable "herd" immunity.

A second test was made in Fulani-owned herds to ascertain that calves of one year of age and less could be immunised safely and effectively, as judged by goat-virus challenge (1,000 M.I.D.s). The details and results are shown in Table VIII. Daily temperature records of these animals were kept from the day of inoculation to eight days after challenge.

A small percentage (not more than five) of animals in these trials was not protected, and this is of little practical significance in view of the fact that lapinized virus can be used on all animals in a herd, whereas even in the presence of outbreaks of rinderpest, calves are not usually given caprinized virus because of its high pathogenicity for them.

Reaction of Inoculated Cattle to Lapinized Rinderpest Virus

Information on this point has been abstracted from the records of unhoused cattle. The records showed that normally the morning (before 8 a.m.) temperature of control or non-immunised cattlle did not reach 102° F. under the conditions of our tests. A significant rise was therefore regarded as one where the temperature reached or exceeded 102° F. on two or more days and clear cut results could then be obtained with each animal. Where reactions are minimal, as with low grade cattle in Kenya, a statistical analysis of large groups may be necessary to establish that any reaction has occurred (Scott, 1954).

Table IX shows details for animals subsequently found immune to challenge. Figure IV illustrates the wide distribution in reaction periods. In calves there is evidence that a temperature rise occurs more frequently and is probably earlier in onset and longer in duration than in older animals. Nakamura *et al.* (1943), Kwong and Lee (1947), Alton (1950), Watson (1950), all had similar experiences.

In 21 of the 45 animals which showed pyrexia the peak was 103° F. or above; in six the maximum recorded was 104° F., or above, and of these four were calves which showed pyrexia of eight or more days' duration. No other clinical sign attributable to lapinized virus was observed.

Discussion

Figure IV for lapinized virus may be compared with Figure I, the temperature reaction of the same type of cattle to goat-adapted virus. The latter gives a distribution which is obviously normal and points to a homogeneity in the cattle population, so far as their susceptibility to the virus is concerned. With lapinized virus, in both adult and young animals, there is a very wide variation in the intensity and duration of reaction. We have noted the failure to immunise about 5 per cent of cattle given the latter product in a dose of 2 mg. (about 1,000 M.I.D.s) and the 25 per cent failure using 100-200 rabbit M.I.D.s. Accurate titrations of vaccine cannot be carried out in such cattle, as a basic assumption is that the animals inoculated should, so far as possible, be of uniform susceptibility. Others have put forward a comparable explanation to account for similar aspects of the behaviour of lapinised virus in other breeds of cattle. Fukusho and Furuva (1953) did so, even when working with hypersusceptible, Japanese Black cattle which

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could only be immunised safely by this virus if hyperimmune serum was given simultaneously. Stevenin *et al.* (1955) reported no detectable reaction in some asiatic buffaloes whilst other similar animals showed severe reactions and even died. Brown *et al.* (1955), describing unusually severe reactions in a herd of high-grade Guernsey cattle in Kenya, stated that no obvious cause for the apparently abnormal susceptibility could be discovered. Kwong and Lee (1947) recorded similar great variability in the response of European dairy cattle.

A similar phenomenon is encountered with other viruses which, by progressive attenuation are reaching the limit of infectivity for a particular species. Sabin *et al.* (1954) found that poliomyelitis virus strains, attenuated by rapid passage in tissue cultures, soon reached a point where no accurate titration was possible in cynomolgus monkeys. Henderson (1949) also found that it was not possible to compare accurately the titres of bovine strains of foot-and-mouth disease virus by intradermal inoculation of guinea-pigs; the converse was also true. He concluded that " if accuracy is required . . . the virus must be titrated using the same species of animal as that for which the strain is adapted ". These observations have an obvious practical importance for those dealing with attenuated rinderpest viruses. The minimum dose of lapinised virus to immunise, for instance, a very large proportion of zebu cattle can be determined, but accurate titrations cannot be carried out in this species; they must be performed in a host-system of uniform high susceptibility and this, at present, means rabbits. It is possible that similar consideration apply to the more highly attenuated, egg-adapted strains of rinderpest virus. In this case there is an added difficulty, that titrations cannot readily be made and there is no highly susceptible, alternative host.

The work reported here shows that lapinized virus can be effectively used to immunise West African (Fulani) zebus. Its disadvantages are associated, in the main, with its greater fragility. However, Piercy (1953) produced evidence that the vaccine, if properly prepared, would withstand storage at 0—4° C. for up to 12 weeks without a dangerous fall in potency. The diluent used for lyophilised vaccine should be chilled and buffered and resuspension must be carried out thoroughly and the inoculations completed within 30 mins. (Scott and Brotherston, 1952). If these conditions could be ensured there is little doubt that rinderpest immunisation would be a more popular and less hazardous undertaking in parts of Nigeria.

A second disadvantage is that it is difficult for the field veterinarian to keep a check on the efficacy of any particular inoculations. With goat virus this is easily done by recording the temperature of representative groups of animals for about a week; with lapinized virus it is manifestly impossible, since less than 30 per cent of adult zebu cattle show easily detectable reactions. For greater security in field practice it would be desirable to increase the standard dose of dried vaccine to 10 mg. and thus allow for a fall of virus content to one-fifth of its original potency, whilst still ensuring 95 per cent immunity. This latter dose is the one which Mornet *et al.* (1954) regarded, largely on empirical grounds, as a " vaccinal " dose and is somewhat higher than that advocated by East African workers, who generally used 4 mg., i.e., about 20 M.I.D.s minimum (Brotherston

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and Purchase 1952, Scott, G. R. 1952). Daubney (1951) referred to variability between batches of dried vaccine and noted that .5 g. of " poor batches produced immunisation of more than 88 per cent of cattle inoculated ". He also noted that the I.D.50 for Cyrenaican or Egyptian cattle was approximately 100 times that for rabbits, which seems to imply very low titres of virus in his preparations.

In the Annual Report of the Department of Veterinary Services, Kenya (1953), it was stated that the vaccinal dose of caprinized virus had been increased from 4 mg. to 8 mg. (i.e., 40 " immunising " doses) following the observation that batches of vaccine could only be expected to immunise 100 per cent of the cattle inoculated at dose of 1/5000 g. No similar figures appear to have been published for lapinised virus.

In an effort to find another alternative to goat virus for immunisation of Nigerian cattle we have recently turned our attention to egg-adapted strains, at the level of attenuation where they produce regular and easily detectable temperature reactions of an " indicator " type, without other adverse signs. The preliminary results with a yolksac passage strain, developed in Egypt and received through the courtesy of Mr. R. Daubney, C.M.G., O.B.E., have been very encouraging. In the meantime larger field trials have been initiated in which the duration of immunity following lapinized virus inoculation in our zebu cattle will be determined.

Summary

1. Certain difficulties in rinderpest immunisation in Nigeria are discussed.

2. Data are presented on the reaction of local zebus (White Fulani) to inoculation of goat-adapted virus under favourable laboratory conditions.

3. Lapinized virus produces consistent immunisation of these cattle, and estimations are given for the I.D.50 and " vaccinal " dose of virus-vaccine.

4. The wide variability in reaction of Northern Nigerian Zebus to lapinized virus is discussed in relation to the titration of this and similar attenuated rinderpest viruses in cattle.

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REFERENCES

Alton, G. G. (1950): *Bull. Off. int. Epiz., 33,* 202.
Birkett, J. D. (1953): *Bull. epiz. Dis. Africa, 1,* 314.
Brotherston, J. G. (1951a): *J. comp. Path., 61,* 263.
Brotherston, J. G. (1951b): *Ibid., 61,* 289.
Brotherston, J. G. and Purchase, H. S. (1952): *Brit. vet. J., 108,* 96.
Brown, C. W., Scott, G. R., Brotherston, J. G. (1955): *Vet. Rec., 67,* 467.
Cheng, S. C. and Fischman, H. R. (1948): Rinderpest Vaccines Their Production and Use in the Field. *F.A.O. of U.N.O., Washington, U.S.A.* (1949).
Daubney, R. (1948): Ibid.
Daubney, R. (1951): *Bull. Off. int. Epiz., 31,* 1.
Fulton, A. (1950): *Ann. Rpt. Dept. Vet. Services, Gambia Govt. Printer, Bathurst,* 1951.
Fulton, A. (1951): *Ibid.* Govt. Printer, Bathurst. 1952.
Fukusho, K. and Furuya, K. (1953 a and b): *Govt. exp. Stat. anim. Hyg. Expt. Rpt.,* No. 26 27 and 35.

Fukusho, K., Ishii, S., Takamote, H. (1953): *Ibid., 26,* 35.
Gambles, R. M. (1952): *Ann. Rpt. Vet. Dept. Nigeria.* 1950-51, Govt. Printer, Kaduna, 1952.
Green, A. R. (1951): *Bull. Off. int. Epiz., 35,* 208.
Illartein, P. R. and Guerret, M. (1954): *Bull. Soc. exot., 47,* 422.
Ishii, S. and Takamote, H. (1953): *Govt. exp. Stat. amin. Hyg. Expt. Rpt., 26,* 49 (Abst. in *Vet.* Bull. No. 2737).
Hammond, R. A. (1954): *Ann. Rpt. Dept. Vet. Serv. Kenya,* 1953. Govt. Printer, Nairobi.
Henderson, W. M. (1949): *Agric. Res. Council Sp. Rpt.* Series No. 8. London, H.M.S.O., 1949.
Kwong, F. J. & Lee, C. T. (1947): *Chinese J. Anim. Husb. Vet. Med., 7,* 23 (quoted by Cheng and Fischman (1948)).
Lee, R. P. (1955): To be published.
Lindley, E. P. (1952): *Sp. Rpt. to Director of Vet. Services,* Nigeria.
Mettam, R. W. M. (1943) (1945) (1946) (1951): *Ann. Repts. Vet. Dept. Nigeria,* Govt. Printer Lagos or Kaduna.
Mornet, P., Orue, J., Labouche, C. and Mainguy, P. (1954): *Rev. D'Elev. Med. Vet. Pays. trep., 6,* 125.
Nakamura, J. and Fukosho, K. (1939): *Jap. J. vet. Sci, 18,* 75 (quoted by Cheng and Fischman (1948)).
Nakamura, J., Fukusho, K. and Kuroda, S. (1943): *Jap. J. vet. Sci., 5,* 455.
Nakamura, J. and Miyamoto, T. (1953): *Am. J. vet. Res., 14,* 307.
Piercy, S. E. (1953): *Proc. XVth int. Vet. Cong., Stockholm.*
Plowright, W. (1952): *Brit. vet. J., 108,* 450.
Purchase, H. S., Burdin, M. L., Scott, G. R. and Brotherston, J. G. (1952): *Vet. Rec., 65,* 590.
Reed, L. J. and Muench, H. (1938): *Am. J. Hyg., 27,* 493.
Sabin, A. B., Hennessen, W. A. and Winsser, J. (1954): *J. exp. Med., 99, 55.*
Scott, G. R. (1952): *Vet. Rec., 64,* 137.
Scott, G. R. and Brotherston, J. G. (1952): *J. comp. Path., 62,* 108.
Scott, G. R. (1954): *Bull. epiz. Dis Africa, 2,* 399.
Scott, G. R. (1954): *Nature, 174,* 44.
Scott, G. R., Macleod, W. G. (1955): *J. comp. Path., 65,* 236.
Simpson, S. (1954): *Bull. epiz. Dis. Africa, 2,* 6.
Stevenin, G., Huard, P. and Geoffen, Y. (1955): *Bull. Soc. Path. exet., 48,* 405.
Thorne, A. L. C. (1953): *Ann. Rpt. Vet. Dept.* Nigeria. 1951-52. Govt. Printers, Nigeria, 1953.
Thorne, A. L. C. (1950): Personnal communication.
Watson, H. C. (1950): *Vet. Rec., 62,* 519.
Weller, T. H., Enders, J. F., Robbins, F. C. and Stoddard, M. B. (1952): *J. Immunol, 69,* 645.
Wells, E. A. (1951): *Vet. Rec., 63,* 165.

ON GENETIC DIFFERENCES IN RESISTANCE OF FOWLS TO ENCEPHALOMALACIA

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FROM their interesting study of the incidence of encephalomalacia in fowls, Carnaghan and Markson (1956) concluded that heavy breeds are more susceptible than Leghorns. In the light of previous evidence such a finding was to be expected.

However, with respect to their conclusion, they go on to say "This is the reverse of Howes and Hutt (1952) in the U.S.A. who found with chicks maintained on a vitamin E deficient diet that *the White Leghorns were definitely more susceptible to encephalomalacia than were the heavier Rhode Island Red and Barred Rock breeds.*" The italics are mine.

Actually, what Carnaghan and Markson found is in complete agreement with what Howes and I found. The only item in reverse is their statement concerning our results. These are given in condensed form in Table I.

As these figures show, and as we stated in our summary, "the Leghorns proved to be significantly more resistant than the heavy breeds, whether resistance was measured by the proportion affected or by the proportion that died."

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That Leghorns are more resistant than heavy breeds is further attested by the fact that the condition is seldom encountered in Leghorns under field conditions in the United States, and any trouble that the disease may cause is largely confined to

TABLE I

Incidence of encephalomalacia experimentally induced in chicks of different breeds.

	Heavy breeds				Leghorns			
Series	Breed	Chicks tested, number	Showing symptoms, per cent	Died per cent	Variety	Chicks tested, number	Showing symptoms, per cent	Died per cent
I	R. I. Red	76	91.8	77.6	White Leg.	61	68.9	50.5
II	R. I. Red	39	94.9	64.1	W. Leg. A	37	70.3	43.3
	B. P. Rock	35	91.4	68.6	W. Leg. B	39	79.5	51.3

the broiler flocks. These are almost entirely heavy breeds, or crosses between heavy breeds.

Along with their greater resistance to encephalomalacia, Leghorns differ from heavy breeds by requiring less manganese and less vitamin D. They also utilize thiamine more efficiently and put more of it in their eggs than do hens of the heavy breeds. Leghorns are more resistant to pullorum disease and to extreme heat than are heavy breeds. The only comparison known thus far in which the Leghorns seem biologically less fit than the heavy breeds in that apparently the former are more susceptible to some nematode worms. The literature on all these breed differences is reviewed elsewhere. (Hutt, 1949).

While these differences in physiological efficiency between Leghorns and heavy breeds are clear-cut and significant, no corresponding differences among different kinds of heavy breeds have been proven. Reasons for the biological superiority of the Leghorns are not known.

The fact that breeds differ in susceptibility to encephalomalacia is clear evidence that that susceptibility is genetically determined. This being so, one way to reduce losses from the disease is to breed parent stock (for the production of broilers) that is resistant to encephalomalacia.

American feed manufacturers and nutritionists are now trying to find some substance which, when added to the mash, will protect every last biologically unfit chick against encephalomalacia. Such a procedure is desirable so long as the protection is restricted to birds that are to be marketed, but if it be extended also to chickens raised for reproduction, that use is dysgenic. Inevitably it must encourage the multiplication of the biologically unfit.

An obvious solution to this difficulty is to provide every possible protection for birds that are to be marketed, but for breeding stock to use a diet that would reveal the five or ten per cent. of the flock that are most susceptible. In pedigree stock it should be feasible then to raise the average level of resistance to encephalomalacia by selective breeding.

Among eight sire families of about 120 chicks each reported by Ranby and Outridge (1954), the incidence of encephalomalacia was 23 per cent in one, and 12

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per cent in another, but in all the chicks from the other six sires there were only one of two cases. With such variation among sire families, a little selection should raise significantly the average resistance in a strain.

This is not likely to happen. Feed manufacturers, though eager to provide different formulae for chicks of different ages, for poultry of different species, and even for birds with different destinies in this world, have thus far shown no interest in catering to fowls that differ genetically in their nutritional requirements. Because of this Procrustean attitude, my White Leghorns eat extra manganese, which they do not need, but which lessens the complaints about perosis from the customers who raise New Hampshires. An anti-oxidant (diphenyl-para-phenylene-diamine) found to reduce the incidence of encephalomalacia in heavy-breed broilers had already worked its unwanted way into commercial mashes for our laying Leghorns when, providentially, it was found to cause undesirable side effects in other species and was withdrawn by governmental decree.

REFERENCES

Carnaghan, R. B. A. and Markson, L. M. (1956): *Brit. vet. J., 112,* 140-144.
Howes, C. E. and Hutt, F. B. (1952): *Poultry Sci., 31,* 360-365.
Hutt, F. B. (1949): Genetics of the Fowl. New York and London. McGraw-Hill Book Co.
Ranby, P. D. and Outridge, A. H. (1954): *Queensland Agric. J., 79,* 285-290.

REVIEWS

BIOLOGICAL ASPECTS OF THE TRANSMISSION OF DISEASE. Edited by C. Horton-Smith and published for The Institute of Biology by Oliver & Boyd, Edinburgh and London. 1957. Pp. X + 184. Price 21s.

THIS book contains 22 papers presented by botanists, zoologists, medical and veterinary scientists at a colloquium organised by the Institute of Biology. One sees, therein, an exchange of knowledge and of viewpoint amongst workers in allied biological fields, each contributor adding his quota to the discussion of a topic not only of mutual but also of scientific interest and importance. The subjects and their contributors are: The Dispersal of Fungi Pathogenic for Man and Animals by G. C. Ainsworth, The Dispersal of Bacterial Plant Pathogens by J. E. Crosse, The Soil as a Reservoir of Pathogenic Mico-organisms by S. D. Garrett, Fungal Diseases of Plankton Algæ by J. W. G. Lund, Bacteria of Veterinary Importance and their Transmission by Non-biological Agencies by Professor R. Lovell, Dispersal of Pathogenic Amœbæ by R. A. Neal, Factors Affecting the Transmission of Coccidia and the Development of Disease in Fowls by C. Horton-Smith, The Transmission of Helminths of Veterinary Importance by E. L. Taylor, The Direct Transmission of Disease between two Organisms of the same Species by Professor R. Cruickshank, The Transmission of Viruses from Animal to Man other than by Arthropods by Professor C. F. Barwell, The Transmission of Viruses to Vertebrates by Arthropods by Professor G. W. A. Dick, Insects and the Spread of Fungal Disease by P. K. C. Austwick, The Transmission of Plant Pathogens by Seed by Mary Noble, The Transmission of Plant Viruses by Insects by F. C. Bawden, F.R.S., The Transmission of Trypanosomes and its Evolutionary Significance by C. A. Hoare, F.R.S., Cyclical Transmission of

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Hæmosporidia by Professor P. C. C. Garnham, The Transmission of Schistosomiasis by O. D. Standen, The Transmission of Experimental Filariasis by D. S. Bertram, The Evolution of some Animal Viruses by C. H. Andrewes, F.R.S., Properties of some Infective Agents in Relation to their Modes of Transmission by Professor W. I. B. Beveridge, The Dissemination of Plant Diseases and Pests in International Plant Trade by W. C. Moore and The Population Dynamics of the Filariæ of Man with particular reference to *Loa loa* and *Onchocerca volvulus* by W. E. Kershaw. The discussion of the various contributions and an author and subject index occur at the end of the book.

THE LIFE OF MAMMALS, by J. Z. Young. Oxford University Press. Price £4 4s. 0d.

THIS book has been put together with a very definite philosophical outlook, which in truth, dissipates the drudgery of the complete factual and adds a new interest to the somewhat inert mammalogy. A successful attempt has been made to weld structure with function and also to introduce here and there, where reasonably possible, " snippets " of the developmental and ancestral lore. The combination of these essentials in this manner gives a picture of the living organism. Each successive chapter portrays a realistic sequence of events in an interesting and simplified scientific manner and builds the mammal into a living whole.

The student, particularly the veterinary one, will find a " facile " application of terminology, absence of unessential detail and an approach to the all important preclinical subjects which is without tedium. In a relatively short space of time a vast field of work is pleasantly and " understandingly " covered. The blending of subjects is used so skilfully that it gives support to the idea that this is the ideal teaching method. The co-ordination of anatomy and physiology looks so simple, but to attain so smooth a sequence of events requires much groundwork of dissection and experimentation that it is only one of great experience and knowledge who could combine and apply them so aptly.

The chapters on the nervous system are conceived and executed beautifully and give an intimate account of an intricate and ponderous subject in a lucid and concise manner.

Every one of the forty-seven chapters is intriguing and there is a flow and continuity between each creating an interdependence so essential for the assimilation of the whole. The elaborate means of control of each living system, the adaptations throughout the generations of time, the devices to suffice the environmental, prove the mammal to be a homeostatic machine of the most delicate mechanism. This could not be shown better. The last chapters on the developmental process complete the biological picture and are of great value.

The Life of Mammals deserves a high place in the list of books recommended to veterinary students, and it would illuminate the shelves of a graduate's library. The summary of the contents is planned well and leaves one in no doubt where to seek and find what is wanted. The print is of good form and size and makes the reading comfortable and easy. There are more than three hundred drawings and

diagrams, which can be followed without difficulty, illustrating the context well. The bibliography of the most modern references on each chapter is adequate even without the classical references.

THE NATURE OF BRUCELLOSIS, by Wesley W. Spink. Minesota University Press. London: Oxford University Press. 1956. Pp. 464+xii. Many figures. 63s. net.

THIS is the biography of a disease and the autobiography of a laboratory and clinic which have been devoted to a study of infections caused by Brucella. A critical account of the diseases which constitute Brucellosis in man and in animals precedes the clinical descriptions of 244 human cases of the disease; there are three more appendices which deal with technique, and with two reports of the United States Livestock Sanitary Association. A bibliography with over 900 references and a subject index completes the book.

It is the author's view that the human disease is declining rapidly in incidence in Minnesota and that the disease in animals may possibly be eliminated within the State in the very near future. Many of the lessons learned may be, and have been, applied elsewhere; a further source of satisfaction is the fact that many of the author's young associates have become expert in the methods of research by participation in these studies on Brucella infections.

Brucellosis is an ancient disease and probably existed at the time of Hippocrates. It has an exciting history and this provides an excellent example of the profit accruing to studies which take into consideration the part played by animals as well as man. The story of the evolution of the concept of the disease is told and illustrated with pictures of those who have helped and are helping to understand it more fully. The Brucella cell, its antigenic structure and its dissemination are described and discussed as well as studies on the pathogenesis of the disease, its course and epidemiology. Brucella are intracellular parasites and this form of parasitism influences the tissue reactions in the host, and for this reason the disease is an excellent model for the study of other diseases and of this form of parasitism. Therapeutic measures, preventive measures as well as diagnostic procedures are described also.

The prevention and ultimate elimination of human Brucellosis is dependent upon the eradication of the disease in animals because basically Brucellosis is not a human disease. An account of the nature of Brucellosis is therefore of equal interest to veterinary surgeons and to physicians. This volume contains much of interest and of value to both as well as to public health workers, clinical pathologists and bacteriologists.

NOTICES

WILLIAM R. WARNER & CO., LTD., announce the formation of a new Veterinary Division.

Pacatal—veterinary—a phenothiazine derivative, the first of their new range of veterinary preparations will be introduced at the B.V.A. Exhibition in Cambridge this month.

Pacatal—veterinary is a new drug representing a significant advance in

animal tranquillizing agents. The unique chemical structure of this new drug provides marked pharmacological advantages and is claimed to be without the troublesome side-effects so frequently encountered. Pacatal—veterinary calms the animal without inducing somnolence or affecting alertness. Restless and ill-tempered animals become quiet, are more easily handled and appear indifferent to their surroundings.

Pacatal—veterinary is indicated in a wide range of conditions necessitating control of excitement, unruliness and fear, and as an aid in the reduction or elimination of pain. It is claimed that the drug acts as a potentiator of analgesics and anæsthetics, materially reducing the quantities of those agents required.

This product is available as tablets for oral use—25 mg. tablets in bottles of 50 and 500, 100 mg. tablets in bottles of 50, and in 2 ml. ampoules containing 50 mg. packed in boxes of 10—for subcutaneous, intramuscular or intravenous injection.

MESSRS. A. & G. NICHOLAS, LTD., Slough, Bucks, wish to announce that they are marketing "Megimide" Sodium. Trial samples of this material are now available to members of the veterinary profession. Further details as to the distribution of this preparation will be announced in the veterinary press.

IMPERIAL CHEMICAL INDUSTRIES, LTD., Pharmaceuticals Division, announce that they have placed on their sales range as from August 6 three new intramammary creams for the prevention and treatment of bovine mastitis. The two "Dispolac" Creams are the first readily dispersible formulations of procaine penicillin G and procaine penicillin dihydrostreptomycin creams for intramammary injection.

"Dispolac" P. Contains 100,000 units procaine penicillin G in each tube. Issued in boxes of 12 and 100 tubes.

"Dispolac" P.S. Contains 100,000 units procaine penicillin G with 100,000 units of dihydrostreptomycin sulphate in each tube. Issued in boxes of 12 tubes.

The base of these two formulations is rapidly dispersed in the milk and ensures that the antibiotics reach all parts of the duct system. Effective concentrations of the antibiotic are brought into immediate and intimate contact with the invading organisms. Rapid elimination in 36-48 hours is also an additional advantage.

Procaine Penicillin Intramammary Cream "Avlon" Brand. Each tube contains 300,000 units procaine penicillin G in a non-dispersible mineral oil base. Issued in boxes of 12 tubes. It is stated that this formulation is particularly useful for obtaining a "depot" effect in dry cows.